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ILLINOIS BIOLOGICAL MONOGRAPHS

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EDITORIAL COMMITTEE

JOHN THEODORE BUCHHOLZ

FRED WILBUR TANNER

HARLEY JONES VAN CLEAVE

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TABLE OF CONTENTS

- No. 1. Generic Relationships of the Dolichopodidae (Diptera) Based on a Study of the Mouth Parts. By SISTER MARY BERTHA CREGAN, R.S.M.
- No. 2. Studies on *Gregarina blattarum* with Particular Reference to the Chromosome Cycle. By VICTOR SPRAGUE.
- No. 3. Territorial and Mating Behavior of the House Wren. By S. CHARLES KENDEIGH.
- No. 4. The Morphology, Taxonomy, and Bionomics of the Nemertean Genus *Carcinonemertes*. By ARTHUR GROVER HUMES.

INDEX TO GENERA

For alphabetic list of species used, see pages 8 and 9.

- Achalcus, 14, 16
 Achradocera, 16, 17
 Acropsilus, 15
 Agonosoma, 14
 Anchineura, 16
 Anepsiomyia, 14, 16, 17
 Aphrosylus, 11, 14, 17, 21, 23, 24, 26, 28, 29
 Argyra, 11, 14, 16, 17, 20, 22, 23, 25, 27, 29, 30
 Asyndetus, 14, 16, 17
 Calychochaetus, 16, 17
 Campsicnemus, 12, 14, 16, 17, 20, 22, 23, 25, 26, 28, 30
 Chrysotimus, 14, 16, 17
 Chrysotus, 11, 14, 16, 17, 21, 23, 24, 26, 28, 29, 31
 Coeloglutus, 14, 16
 Condyllostylus, 16, 17, 22, 23, 24, 26, 27, 30
 Diaphorus, 7, 11, 14, 16, 17, 18, 21, 23, 24, 26, 28, 29, 31
 Diostracus, 14, 15, 16, 21, 23, 25, 26, 28, 29, 31
 Dolichopus, 8, 11, 13, 14, 15, 17, 18, 19, 20, 22, 24, 25, 29, 30
 Eutarsus, 14, 16
 Gonioneurum, 15
 Gymnopternus, 11, 15, 17, 22, 24, 25, 26, 28, 30
 Hercostomus, 15
 Hydrophorus, 11, 13, 14, 15, 16, 17, 22, 23, 25, 26, 28, 30
 Hygroceleuthus, 15, 18, 22, 24, 25, 27, 29, 30
 Hypocharassus, 11, 14, 15, 16, 22, 23, 25, 26, 27, 28, 30, 31
 Hyptiochaeta, 16, 17
 Laxina, 20, 21, 23, 24, 26, 27, 30
 Leptocorypha, 15
 Leptorhethum, 14, 16, 17
 Leucostola, 14, 16, 17
 Liancalus, 11, 14, 15, 16, 17, 22, 23, 25, 27, 29, 30
 Lyroneurus, 16, 17
 Macellocerus, 15
 Machaerium, 17
 Medeterus, 7, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 31
 Megistostylus, 16
 Melanderia, 8, 11, 20, 22, 23, 25, 27, 29, 30
 Mesorhaga, 14, 16, 17, 22, 23, 25, 27, 30
 Millardia, 21, 23, 25, 26, 28, 29
 Nematoproctus, 14
 Neurigona, 11, 14, 16, 18, 20, 21, 23, 24, 26, 28, 29
 Nothosympycnus, 14
 Orthochile, 15, 18, 19, 20
 Paraclius, 15
 Paraphrosylus, 15
 Parasyntormon, 14
 Pelastoneurus, 15, 22, 24, 25, 27, 29, 30
 Peloroepodes, 14, 16, 22, 23, 25, 26, 28, 30
 Peodes, 15, 16
 Phylarchus, 14, 15, 16
 Plagioneurus, 11, 14, 15, 22, 24, 25, 26, 28, 30
 Polymedon, 15
 Porphyrops, 14, 16, 18
 Psilichium, 15
 Psilipodinus, 14
 Psilopus, 17
 Rhaphium, 12, 14, 16, 20, 21, 23, 24, 26, 27, 29
 Sarcionus, 15
 Scellus, 11, 13, 14, 15, 16, 21, 23, 25, 27, 29, 30
 Schoenophilus, 15
 Sciapus, 11, 16, 17, 22, 23, 25, 26, 27, 30
 Stenopygium, 15
 Stolidosoma, 16
 Subsympycnus, 16, 17
 Sybistroma, 15
 Symbolia, 16, 17
 Sympycnus, 11, 14, 16, 17, 22, 24, 26, 29, 30
 Syntomoneurum, 15, 16
 Syntormon, 11, 14, 16, 18, 22, 24, 26, 28, 30
 Systemus, 16
 Tachytrechus, 11, 15, 17, 20, 22, 24, 27, 29, 30
 Teuchophorus, 14, 16, 17, 18, 22, 23, 24, 27, 29, 30
 Thinophilus, 11, 14, 15, 16, 17, 20, 22, 23, 25, 26, 28, 30, 31
 Thrypticus, 14, 15, 16, 21, 23, 24, 26, 27, 29, 31
 Xanthina, 14, 16
 Xanthochlorus, 11, 14, 16, 17, 20, 21, 23, 24, 26, 27, 29
 Xiphandrium, 16

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STUDIES ON GREGARINA BLATTARUM
WITH PARTICULAR REFERENCE TO
THE CHROMOSOME CYCLE

WITH SIX PLATES AND
TWO TEXT-FIGURES

BY
VICTOR SPRAGUE

CONTRIBUTION FROM THE ZOOLOGICAL LABORATORY OF THE
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CONTENTS

INTRODUCTION	7
Historical Review	7
Material and Methods	9
Glossary	11
OBSERVATIONS	13
Vegetative Phases	13
Reproductive Phases	13
Encystment	13
Shape and Size Variation of the Cysts	15
Gross Features of Cyst Development	17
Mechanism of Sporoduct Eversion	20
The Mature Spore	22
Nuclear Phenomena	24
DISCUSSION OF CHROMOSOME BEHAVIOR IN GREGARINES	32
Types of Reduction	32
Cephalina	32
Acephalina	33
Synopsis	35
Haploid Forms	35
Diploid Forms	36
Number of Meiotic Divisions	37
Haploid Forms	37
Diploid Forms	38
General Considerations	39
SUMMARY AND CONCLUSIONS	40
LITERATURE CITED	43
PLATES	45

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INTRODUCTION

THE SUBJECT of these studies, *Gregarina blattarum* von Siebold, is one of the most common and widely known of the Sporozoa and has been studied by many investigators. Many phases of its life-cycle have, nevertheless, received but scant attention and are not at all understood. One particularly neglected, but very significant, period is that which involves syngamy and the meiotic phenomena. The present investigation deals principally with that period but also includes an account of the process of encystment and the development of the cyst.

HISTORICAL REVIEW

A number of early workers probably saw *Gregarina blattarum* before it was actually described but did not recognize its true nature. Among them was von Siebold (1837), who observed ovoid bodies in the gut of *Blatta orientalis* Linnaeus, which he thought were insect eggs. At that time he noted their resemblance to *Gregarina ovata* Dufour, 1828, the first gregarine ever to be named and described. Later, after observing the ovoid bodies in locomotion, von Siebold (1839) changed his earlier view and concluded that they were peculiar helminths. He then gave the original description, including observations on morphology, locomotion, and syzygy, and proposed the name of *Gregarina blattarum*.

The next contribution was made by Stein (1848), who collected feces from *Blatta orientalis*, recovered mature cysts from them, and described the spores of *Gregarina blattarum*. He wrote that the spores were "tonnenförmig, 1/200'" lang und 1/570'" breit, sehr blass, aber von starken Conturlinien begrenzt . . . Schale und Inhalt liessen sich nur bei sehr starken Vergrößerungen von einander unterscheiden." In the gut of one roach two mature cysts were found which Stein thought had been ingested with the food. One of them was ruptured and spores were seen to emerge from it. Among spores Stein observed some very young gregarines which, because of their very small size, he believed to have escaped from the spores.

Leidy (1853) characterized the genus and, not having access to the writings of von Siebold and Stein, described in detail what he believed to be a new species of gregarine from *Blatta orientalis*, calling it *Gregarina blattae orientalis*. The description included the first observation on longitudinal myonemes in gregarines. This observation was confirmed by Lankester (1863).

Schneider (1875), who gave an excellent summary of the literature on gregarines, made extensive observations on the minute structure of a

number of species and introduced a very large portion of our modern terminology. He also described and figured, for the first time, a cyst of *Gregarina blattarum* in the process of discharging its spores.

Bütschli (1881) observed the process of encystment by placing two individuals in egg albumen which, according to Watson (1916), is "a process never before seen and very rarely described since." Bütschli further studied the spores and the trophozoites of this organism and recognized, for the first time, the epimerite. It appears probable that Bütschli, as he himself wrote, was first to perform infection experiments with gregarines.

While Bütschli studied cysts in the fresh condition, Wolters (1891) used sections in his observations on the development of the cyst. He also studied the nucleus and the ectoplasmic layers of the sporodin in considerable detail.

Marshall (1893) repeated the observations of Bütschli and, in addition, made a study of the nuclear changes, including those in the developing spore. This author discovered the cross-striations in the ectoplasm of *Gregarina blattarum*.

In 1903 Crawley reported the occurrence of *Gregarina blattarum* in *Periplaneta americana* (Linnaeus) and *Blattella germanica* (Linnaeus) as well as in the original host.

Another study of the nuclear changes was made by Schiffmann (1919), who wrote, "*Gregarina blattarum* ist seit der Erkenntnis der geschlechtlichen Vorgänge noch nicht bearbeitet worden." Schiffmann outlined the general course of development but was unable to demonstrate much detail, since the section method alone, which was used in that study, probably is inadequate for revealing the more minute details of nuclear phenomena.

A short time later Jameson (1920) published a paper on *Diplocystis schneideri* Kunstler of far-reaching importance with regard to the chromosome cycle of gregarines. He found, to use his own words: "The sporoblast is a zygote. Its nucleus is formed by the fusion of the two gamete nuclei, each containing a similar set of three chromosomes. The first nuclear division of the sporoblast is a reduction division. From the spireme, which is formed during the early prophases, six chromosomes arise. These lie on an indistinct achromatic spindle and separate into two homologous groups of three, one set of three passing to each pole. A second and a third mitotic division then take place—each presenting the same number (three) of chromosomes—giving rise, finally, to the eight sporozoite nuclei. The sporozoites thus each contain three chromosomes—a single or haploid group. This is the number present again in all the nuclear divisions—including all the divisions associated with gametogenesis—of the gamont, which is the adult organism into which the sporozoite grows.

"This type of reduction division is new, and it explains in a simple fashion the odd chromosome number so common among gregarines. The number found in every division excepting the first sporoblast division is the haploid number. Only once in the whole life-cycle is the diploid number found, namely, in the first division of the sporoblast. It seems probable that a careful re-examination of gregarine life-histories, paying special attention to the sporal divisions, will reveal this to be the real method of reduction in all."

Noble (1938) has recently studied the chromosome cycle in another acephaline form in which, he believes, reduction occurs in the zygote.

Contrary to the generalization of Jameson, gametic meiosis has been observed in some genera of the Acephalina, such as *Monocystis* (Mulsow, 1911; Calkins and Bowling, 1926; Naville, 1927a) and *Urospora* (Naville, 1927).

Jameson included in his paper a list of all gregarines whose chromosome numbers were known and gave the number for each species. The monumental works of Bélař (1926) and Naville (1931) include a few additional species whose chromosome numbers have been more recently determined.

A small number of studies on the nuclei of gregarines have appeared in recent years, although much attention seems to have been given to the cytoplasm, particularly since 1926 when the admirable researches of Joyet-Lavergne gave impetus to this type of investigation. No noteworthy contribution, so far as the writer is aware, has been made for *Gregarina blattarum* since the work of Schiffmann.

MATERIAL AND METHODS

Cockroaches, *Blatta orientalis*, were collected in the fall of 1939 (September to late November) on the campus of the University of Illinois at Urbana and were kept in the laboratory in battery jars. At first, they were fed a diet of yeast, but later (in December) they were given apples instead. The latter diet has an advantage over the former in that it does not render the detection of the parasites difficult when one examines the gut contents and the feces. Furthermore, after the first part of January, 1940, there was a tremendous increase in the incidence and amount of infection, which may have had some relationship to the diet. The true cause of this increase is at present unknown, but it appears probable that the infection was built up over a period of time due to the close confinement of the hosts in the containers. From time to time cysts were placed in the food, and this was probably also a factor in the increased incidence of infection.

The host insects were examined by extracting the gut in 0.75 per cent

NaCl solution and placing it under a binocular dissecting microscope. Cysts were recovered from feces by immersing in water for a few minutes, crushing gently to avoid damaging the cysts, and picking out the latter with a pipette.

Of 810 roaches, which were examined soon after collection between late September and the middle of December, forty-five individuals or 5.3 per cent were infected. Each of the infected individuals contained from one to twelve sporadins, and a total of two cysts was found. In late November and December a few cysts were found also in the feces.

During January and February about fifty hosts were examined, and 30 per cent were found to be infected. In almost every instance of infection at this time, the mid-gut was literally packed full of parasites in all stages of development from young trophozoites to completely formed cysts. Very frequently the hind-gut also contained cysts; in one instance thirty-one cysts were obtained from this region.

Most of the cysts used in this study were collected from feces (during January and February), since all but the earliest stages are easily obtainable from this source. The feces of about one hundred roaches yielded from two hundred to four hundred cysts daily, until most of the roaches suddenly died from some unknown cause. In some instances the pellets consisted almost entirely of cysts, one such pellet containing fifty-eight normal cysts and about a dozen abnormal ones. Frequently large masses of gregarines in the vegetative stages were also noticed in the feces.

After the cysts were collected, it was necessary to keep them under conditions suitable for their normal development in order that desired stages might be obtained. In attempting to do this, several methods were tried and only one was successful. Although cysts air-dried or kept in 0.75 per cent NaCl or distilled water failed to develop, highly successful results were obtained by incubating them, at room temperature, in moist chambers constructed in the following manner: Several layers of circular pieces of towel paper were placed in the bottom of a finger bowl and moistened with water. On top of the towel paper was placed a small piece of black drawing paper. A watch glass containing cysts, without any water, was placed on top of the black paper; and the finger bowl was covered with a glass plate and sealed with vaseline. The cysts were examined from time to time with a binocular dissecting microscope and reflected light, the white cysts appearing in sharp contrast on the black paper in the background.

Cysts were removed from the moist chamber after various intervals of time, depending on the stages desired, and smear preparations and sections were made. The sectioning was done individually or in mass after fixation in the solution of Carnoy, Schaudinn, Bouin, Flemming (strong),

Zenker, Feulgen (sublimate-acetic), or Perenyi. None of these fixatives proved quite satisfactory. All of them penetrated the cyst membranes very slowly, and all except Flemming caused the majority of the cysts to explode. Flemming seemed to penetrate fairly well, but subsequent staining was unsatisfactory. Some fair preparations were obtained after Perenyi, and in instances of good fixation with Carnoy the staining was usually excellent. Sections were stained with Heidenhain's haematoxylin or subjected to the Feulgen nucleal reaction. The best section preparations were obtained by the Carnoy-Heidenhain, Carnoy-Feulgen, and Perenyi-Heidenhain combinations. Sections were found useful for determining the general course of development within the cyst but not good for nuclear detail.

For observations on the nuclear changes the smear method was used almost entirely. Smears were prepared by crushing the cysts on cover glasses, fixing in Schaudinn, Flemming, or Zenker, and either staining with Heidenhain, Giemsa, or Flemming's triple, or subjecting to Feulgen's nucleal reaction. Lang's (1936) formula was used exclusively for the mordant before haematoxylin. The best results were obtained with the Zenker-Heidenhain combination and Schaudinn followed by Heidenhain, Feulgen, or Giemsa.

For studying the process of encystment, associated pairs of gamonts, which showed by their rotating movements that they were ready to encyst, were placed in fresh egg albumen on a depression slide and covered with a cover-glass. Thus, the experiment of Bütschli, who also used egg albumen, was easily repeated a number of times. Attempts to obtain encystment in 0.75 per cent NaCl were unsuccessful, although the gregarines appeared to make strenuous attempts to encyst for an hour or more. Possibly a quite viscous medium is essential for the successful completion of this process, although various unknown factors may be involved.

GLOSSARY

To avoid confusion in terminology, the following glossary is given. Some of the terms are probably new. Others have been more frequently used with regard to the metazoa, and most of them have been applied with various meanings to the gregarines and other protozoa.

Acephaline gregarine: A non-septate form; the body is not divided into protomerite and deutomerite.

Association: Two or more individuals in syzygy.

Basal disc: A hyaline, circular area in the sporoduct membrane surrounding the proximal end of the sporoduct.

Cephaline gregarine: A septate form; the body is divided into protomerite and deutomerite.

Cyst: A spheroidal body, surrounded by a resistant membrane, into which the associated gamonts develop at the beginning of the reproductive process.

Cyst membrane: The elastic, hyaline, laminated covering of the cyst.

Deutomerite: That portion of the sporadin posterior to the septum in cephaline gregarines.

Epimerite: A process at the anterior end of the protomerite by which the young trophozoite is attached to the host cell.

Gamont: The initial stage in gamete-formation; one of the individuals in an association, or in a young cyst, destined to form gametes.

Gelatinous layer: A thick, amorphous, hyaline layer of gelatinous consistency surrounding the cyst membrane.

Meiocyte: Any protoplast in which meiosis is initiated; the zygote or sporoblast in haploid gregarines.

Meiotic division: Any nuclear division involving the segregation of maternal and paternal chromosomes.

Metagamic divisions: Nuclear divisions following syngamy.

Mucoid sheath: A layer of adhesive substance covering the spore.

Myonemes: Contractile fibrils in the ectoplasm of the sporadin.

Perinuclear vesicle: A chromophobic area surrounding the nucleus of the gamete and apparently bounded by a delicate membrane.

Pregametic divisions: The series of nuclear divisions producing the nuclei of the gametes.

Primitive: The anterior individual in an association of gamonts.

Protomerite: The anterior compartment of a septate gregarine.

Protoplast: A cell or any morphologically comparable unit.

Residual mass: Used here with particular reference to that viscous part of the former gamont which remains after the gametes have budded off the periphery.

Satellite: The posterior individual in an association of gamonts. There is usually only one, but more may be present.

Septum: A transverse partition dividing the sporadin into anterior and posterior compartments, the protomerite and deutomerite respectively.

Sporadin: A trophic individual after detachment from the host cell.

Spore: The body into which the sporoblast (zygote) develops.

Spore membrane: The resistant covering of the spore; sometimes called the "sporocyst."

Sporoblast: The initial stage in spore formation. In gregarines it is synonymous with zygote.

Sporoduct: A tubular structure continuous with the sporoduct membrane and serving to conduct the spores out of the cyst.

Sporoduct membrane: A membrane lying just beneath the laminated cyst membrane and containing the sporoducts.

Sporozoite: One of the eight falciform bodies developed within the spore.

Syngamy: Fusion or copulation of isogamous or anisogamous gametes; fertilization.

Synkaryon: Zygote nucleus.

Syzygy: An end-wise association of two or more sporadins.

Trailing chromosome: That chromosome which characteristically follows behind the other members of the chromosome complex in the anaphase. This has sometimes been inappropriately called the "odd chromosome."

Trophozoite: An individual in the feeding stage, either before or after the loss of the epimerite.

Zygote: The body resulting from syngamy; the sporoblast in gregarines.

Zygomeiosis: Chromosome reduction taking place immediately after syngamy in contrast to the usual meiosis which is delayed until gamete-formation.

OBSERVATIONS

VEGETATIVE PHASES

MUCH has been written on the trophic stages of *Gregarina blattarum* by von Siebold, Leidy, Schneider, Bütschli, Wolters, and Marshall. Little can be added to that subject now without undertaking intensive studies of such aspects of the problem as cytology, physiology, and statistical analysis. Any observations made on the vegetative stages in this study are only incidental, but a few of them seem worth noting.

It was observed in a number of hosts having light infections that the sporadins were unusually large. One such individual, found alone in the host, measured about 950 μ long and 550 μ wide (Fig. 1). There may be a relationship between the number of parasites and their size, although conclusive evidence on this question is lacking.

Also of interest is the fact, previously noticed by many workers, that the gregarines often become associated in pairs very soon after the loss of the epimerite. Thus individuals of all sizes, from very small (Fig. 2) to very large, are seen in association. The two members of the association are usually very similar in size, but frequently the satellite is much smaller than the primite. The reverse size relationship, as von Siebold observed, appears never to occur. These associations with unequal individuals form young cysts containing two unequal gamonts (Fig. 15). Cysts of this type provide a method of identifying primite and satellite after encystment and suggest a method whereby the resulting gametes may be studied for possible differences in structure and behavior. Sometimes two small satellites are seen on one large primite, as both von Siebold and Marshall observed (Fig. 3). The two satellites may be approximately the same size, in which case the resulting cyst contains one large and two equally small gamonts, or the two satellites may differ in size. In the latter case, the resulting cyst contains three gamonts of different size (Fig. 12). These unusual types of cysts seem to develop normally and produce typical spores.

REPRODUCTIVE PHASES

Encystment

The process of encystment and subsequent development of the cyst were so well described and illustrated by Bütschli that it is necessary here only to outline that process and mention certain new observations.

In January and February, when abundant material was on hand, hundreds of individuals, in all stages of development, were often found in the mid-gut of a single host. Many of the larger pairs were undergoing characteristic rotating movement indicating imminent encystment.

Pairs of this type, when placed in fresh, undiluted egg albumen, as described above, are easily observed while they continue the process of encystment. The two individuals glide slowly forward, both bending in the same direction, with much folding of the body wall, so that there is a tendency to move in a circle (Fig. 4). The satellite seems to be the more active of the two. While the anterior end of the primite bends to one side, the satellite pushes forward and, at the same time, brings its posterior end up toward the anterior end of the primite (Figs. 5 and 6). When these two ends are brought together, often after many unsuccessful attempts, they adhere and the pair continues to rotate (Fig. 7).

The coming together of the opposite ends of the pair apparently is not accomplished solely by active bending of the bodies. The force of the forward movement seems also to be a factor, since it tends to bend the bodies passively when the anterior end meets resistance in the medium. The bending process seems to be greatly facilitated when the protomerite of the primite collides with bits of debris in the medium, which retard the forward movement. It appears probable also that the viscosity of the medium is a factor in the passive bending. The gamonts, as stated previously, never seemed quite able to make the two ends meet when they were placed in 0.75 per cent NaCl solution, although they frequently underwent active bending and forward movement for more than an hour. If the pair, however, was placed in this solution after adhesion of the opposite ends was completed, encystment proceeded in the normal manner. In the egg albumen, on the other hand, the gamonts were able to accomplish the process from the very beginning. This may be due, at least in part, to the fact that this medium is viscous and resists the forward movement, thus supplementing the active bending of the bodies. It should be noted also that the normal habitat of this parasite is a viscous medium, the gut contents, containing much debris. It seems probable, therefore, that a viscous medium is a mechanical factor necessary for encystment, although other factors are undoubtedly involved.

When encystment occurred in egg albumen *in vitro*, it was observed that frequently no gelatinous layer could be detected outside the true cyst membrane. This layer may actually have been absent, or it may have been indistinguishable from the egg albumen.

About forty-five minutes after the beginning of encystment the true cyst membrane is being secreted, and at this time the young cyst is almost perfectly spherical in shape (Fig. 8). Then the myonemes, which are seemingly in a state of contraction during the early stages of encystment, probably relax, for the young cyst rather suddenly begins to elongate in a direction perpendicular to the plane separating the two gamonts (Fig. 9). The spherical shape of the cyst thus changes into that of a prolate spheroid. While elongation slowly continues for about thirty

to forty-five minutes, the plane between the two individuals becomes somewhat oblique to the long axis.

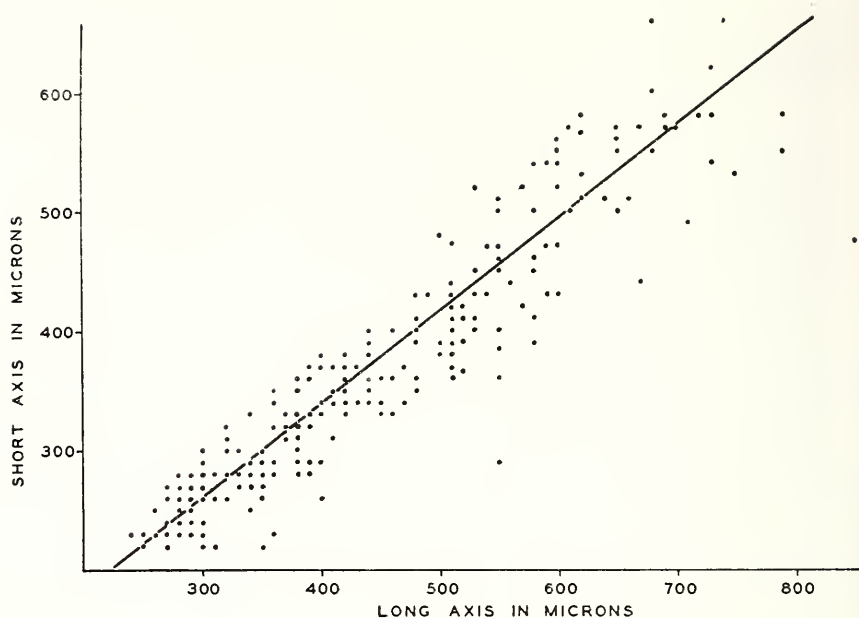
Shape and Size Variation of the Cysts

Bütschli called attention to the change in shape of the young cyst, remarking that it gradually assumes an ovoid form "welche die ausgebildeten Cysten stets zeigen." This author apparently did not observe a sufficient number of cysts formed under normal conditions to notice that they are, on the average, much less elongated than those formed under experimental conditions. If one observes a great number of the former, some are seen to be very long, others almost perfect spheres, and the majority are between these two extremes.

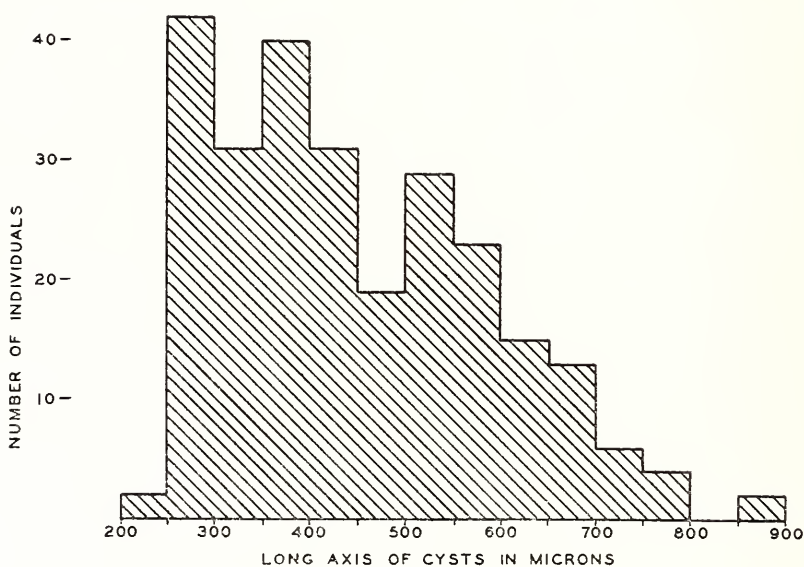
In order to determine mathematically whether there is a relationship between the shape of the cyst and the conditions under which encystment occurs, the following procedure was followed: twenty-five cysts formed within a single host gut in the normal manner were obtained, and fourteen pairs of mature gamonts from the same host were allowed to encyst in egg albumen. The cysts of the two groups were measured, and the ratios of the long and short axes were determined and averaged for each group. The average ratio for the former group was found to be 1.22, the range being from 1.1 to 1.4. The ratio for the latter group averaged 1.76 and ranged from 1.3 to 2.0. These figures seem to be significant. They apparently indicate that an important factor in causing the extreme variation in shape is the immediate surroundings at the time of encystment. Cysts of the spherical type are probably formed in such close confinement that elongation at a certain critical time is mechanically impossible. Longer ones probably are not so closely confined during formation, and thus are able to expand upon the relaxation of the myonemes of the gamonts while the cyst membrane is still in a plastic condition.

Marshall observed some pairs in syzygy which were long and thin and others which were short and compressed. He thought that this fact explains the difference in shape of the cysts, elongated ones developing from the former and spherical ones from the latter.

The membrane of an extremely long cyst has a noticeable lack of uniformity in thickness, being much thicker at the ends than in the middle region (Fig. 11). This can probably be attributed to the fact that the extreme elongation inhibits the normal rotating movements of the individuals within the cyst, thus preventing an even distribution of the substance secreted to form the membrane. Such cysts do not often develop to maturity, for the membrane usually ruptures at the thin places. Cysts with ruptured membranes seem never to continue their development.



TEXT-FIG. I.—Scatter diagram showing a positive correlation between the long and short axes of a random sample of 259 cysts.



TEXT-FIG. II.—Histogram showing size distribution, based on the long axis, of a sample of cysts.

To obtain further information on the variation in shape, 259 cysts in a random sample were measured in microns; and the long axes were plotted against the short axes (Text-fig. I). The points on the resulting figure are seen to fall about evenly on the two sides of a straight line running through them. (A number of the points represent two or more individuals.) This distribution indicates that there is a definite relationship between the long and short axes regardless of the size, all ranges of variation in shape being found in each size group. In other words, there is a positive correlation between the long and short axes, the one tending to vary in direct proportion to the other.

Having demonstrated a straight line relationship between the two axes of the cysts, it is now possible to compare accurately the sizes on the basis of either of the two dimensions. Accordingly, the size groups (based on the long axes) were plotted along the abscissa, the frequencies were plotted along the ordinate, and a histogram was constructed showing the size distribution (Text-fig. II). An extremely rapid rise in frequency is shown, plainly indicating that encystment seldom occurs until the gamonts have attained a rather definite minimum size. This confirms the opinion of Watson (1916) who stated that "only the large individuals in any case may be expected soon to form cysts." Size is thus correlated with gamont maturity, although suitable physiological conditions must undoubtedly exist before encystment can take place. The histogram further shows, as one would expect, a tendency toward a gradual decrease in frequency, while size further increases, suggesting that encystment may be delayed by various physiological factors after mature size has been attained. At two points there are sudden drops in frequency which are difficult to interpret. There are a number of possible explanations, although it must be admitted that none of them is demonstrated. It is altogether possible that, in view of certain nuclear phenomena described later, two or more varieties of the organism are represented. Furthermore, the sample may not be representative or sufficiently large. Again, some of the irregularities may be due to large cysts formed by three or more individuals. And, finally, there are many unknown physiological and environmental factors involved.

The results of the present incomplete investigation suggest the desirability of a thorough-going statistical study in an attempt to discover some of the factors contributing to the size variation of the cysts of *Gregarina blattarum*.

Gross Features of Cyst Development

Changes occurring in the cyst from the time of its formation to the appearance of gametes on the periphery were not observed. Bütschli found that the boundary between protomerite and deutomerite disap-

peared within three hours. The next change he noticed was the appearance of gametes (which were considered by Bütschli and other workers to be young spores) on the periphery about sixteen to twenty-four hours later. In the meantime profound changes in the ectoplasm layers of the gamonts have taken place. These layers have disappeared, in a manner which is not clear, and in their place a layer of gametes has appeared. The observations of the present author on the course of further development will now be described.

The peripheral layer of gametes has been aptly described as resembling a layer of columnar epithelial cells, for they are so crowded together that they have a columnar appearance (Figs. 12 and 16). This gamete layer is very translucent and contrasts sharply with the opaque granular mass which it encloses. Each residual mass is completely covered by the gamete layer, although Bütschli stated: "Auf der Vereinigungsfläche der beiden encystirten Individuen scheint es wohl sicher nicht zur Bildung von Sporen zu kommen." It is difficult to understand why Bütschli failed to see the gametes, which he mistook for spores, on the contiguous sides of the two individuals, for they are as conspicuous there as elsewhere and are seen not only in living material but also in sections. Illustrations of Marshall also seem to show this condition. It is, in fact, these gamete layers, and nothing else, which separate the two individuals at this time and prevent their fusion. As was stated above, the fate of the original ectoplasmic boundaries seems to be, for this species at least, unknown.

Before the gametes begin to separate from the residual mass, the two halves of the cyst contents fill the cyst entirely and are so pressed together that their contiguous sides are flat surfaces (Fig. 16). The next change is a slight rounding up of the two masses so that a space filled with aqueous fluid appears between them. At the same time, gametes begin to separate from the periphery, round up, and pass out into the aqueous fluid (Fig. 17). The first gametes to separate are those at the edge of the flat surface because, perhaps, they are under a smaller amount of pressure than the others which are closely pressed against the cyst membrane. In the cases observed it was often evident that activity appeared in one end slightly sooner than in the other. This may or may not indicate a sexual difference. As was suggested above, it may be possible to answer this question by observing a cyst formed from a large primitive and a small satellite, in which case either of the encysted gamonts can be identified.

As the gametes begin to separate from the edge of the flat surface, each central mass becomes slightly more rounded, thus releasing pressure between the two masses. Consequently, more and more of the gametes in

that region break off. These gametes seem always to move outward toward the cyst membrane and then toward the end of the cyst.

As indicated above, the peripheral layer of gametes around each residual mass seems to act as a cellular membrane which, while intact, encloses that mass and prevents its fusion with the other. When this membrane breaks down, however, the residual masses are no longer confined and they begin to flow into one another with long, irregular extension of their substance (Fig. 18). The flowing together of this substance in the middle region releases pressure on the ends and the gametes in those regions immediately pinch off and pass out into the clear liquid. Whereas the movement of gametes was previously from the middle region toward the ends, there is now a reversal of direction for a short time; but soon no constancy of direction is seen. About ten minutes later the two residual masses have fused into a single viscous mass surrounded by a watery liquid containing gametes (Fig. 19).

The cause of these activities is quite unknown but it may be permissible to suggest an hypothesis. The layer of gametes surrounding the viscous inner mass, which is a colloidal suspension, may permit, or actively cause, the passing through of water while retaining the suspended materials. This loss of water from the inner mass results in a decrease of size and a consequent tendency of the mass to round up. The gametes then pinch off and float out into the watery liquid. The two residual masses, now being in close contact, fuse into one.

After a time, not accurately determined but certainly nearly an hour, the aqueous fluid containing gametes and the residual mass, being no longer separated by any sort of boundary, merge into a single mass which is uniformly granular and fills the entire cyst (Fig. 20). Possibly the pairing of gametes occurs while they are free in the aqueous medium, but this point was not determined.

No conspicuous changes are noticed until the next day when the basal discs of the sporoducts are plainly seen (Fig. 21). These structures are just as Bütschli described them, consisting of circular areas free from large granules like those all around, but containing very small granules which radiate out from the center. In the center is the lumen of the sporoduct. It is at this time that the sporoducts are most easily counted. If the cysts are placed on a slide and rolled with a sidewise pressure on the cover glass, the sporoducts can be counted with a fair degree of accuracy. By this method the sporoducts of a random sample of twenty-four cysts of all sizes were counted. The number was found, as Bütschli observed, to be roughly in proportion to the size of the cyst, varying from five to thirty. The average was thirteen. Bütschli gave the number as three to about a dozen, and Watson gave eight to ten.

During the next two days the only noticeable change is a decrease in the opacity of the cyst, as observed by Bütschli, which seems to be due to a decrease in the paraglycogen content and also to the translucency of the spores in the center. Sometimes the cyst becomes so translucent that the individual spores can be plainly seen within. Then, about forty-eight hours after the sporoducts are first seen, the latter break through the cyst membrane (Fig. 22), and the spores are discharged in long chains. The time given here agrees with the observation of Bütschli, but Schiffmann found that the sporoducts are everted in seven days. It is doubtful whether the moist chamber used by the latter author was of a type to provide the best conditions for development, for the surface of the cysts in that chamber became irregular by the time the sporoducts were first seen. In the present study the external appearance of the cysts seemed to remain absolutely unaltered up to the time of maturity.

Sporoducts were studied by Stein, Schneider, Bütschli, Schiffmann, and others. The observations of Bütschli were particularly thorough, and nothing can be added to his description at this time. The sporoduct is a tapering tube about 200 μ long and with a bulbous enlargement near its base (Figs. 22 and 25). This tube is continuous with a thin membrane, which Bütschli has called the sporoduct membrane, just inside the cyst membrane proper. The time and manner of formation of the sporoduct membrane seems to be completely unknown.

Mechanism of Sporoduct Eversion

The writer is not aware that anyone has yet explained how it is possible for the delicate sporoduct to make its way through the thick, resistant cyst membrane. In the present study certain observations were made which seem to provide an explanation of that phenomenon. When a cyst approaches maturity the basal disc of the sporoduct seems to become slightly raised above the surrounding surface of the sporoduct membrane, forming a small convex protuberance (Fig. 23). The cyst membrane at this point is slightly thinner than elsewhere and may also be raised above the adjacent surface. The thin area in the membrane is very conspicuous when a sporoduct fails to break through due to the release of the internal pressure when other sporoducts are everted (Fig. 24). If one is so fortunate as to be observing the cyst at exactly the right moment the basal region of the sporoduct is seen to burst suddenly through the weakened area in the cyst membrane; and, by rapidly turning inside out, the sporoduct becomes completely extended to the outside. A few droplets resembling oil globules then pass out of the sporoduct and are immediately followed by the spores, which are rapidly discharged

in long chains. The oil droplets possibly serve as a lubricant to minimize friction between the sporoduct and the spores; for the latter, after their discharge, are actually covered by an oily film.

When the thin areas in the cyst membrane were first observed, it was suspected that a lytic action occurs in the region of the basal disc. To obtain information on this question various pH indicators were added to water containing cysts which were ready to discharge their spores. These indicators were unable to penetrate the intact cyst membrane, but they did reach the inside of the cyst when some of the sporoducts were everted. Upon the addition of neutral red the basal disc and the proximal portion of the sporoduct became very dark red, the color being much less intense in the distal region (Fig. 25). A number of dark red granules were also seen in the sporoduct membrane, and others were scattered throughout the interior of the cyst (Figs. 24 and 25). Brom-thymol blue gave comparable results, the color obtained being yellow instead of red. No color changes were noticed with methyl red, phenol red, or brom-cresol purple. On the basis of the color changes with the indicators, it is concluded that the reaction in the basal region of the sporoduct is decidedly acid.

These observations lead to the following hypothesis, which is offered to explain the mechanism of sporoduct eversion: Since acid is present in the basal portion of the sporoduct, there may also be an enzyme in that region acting in the presence of the acid to dissolve, and therefore weaken, the adjacent area of the membrane. The latter, being very elastic, constantly exerts a relatively enormous pressure on the contents within, and, after the enzymes have acted sufficiently, the pressure on the contents forces the sporoduct out through the weakened area. The great pressure exerted by the elastic membrane also adequately accounts for the force which expels the spores.

The elasticity of the cyst membrane is easily demonstrated in a number of ways. If a normal cyst is ruptured, the contents are expelled; and the cyst immediately decreases in size. At the same time, the membrane, which was formerly thin and homogeneous in appearance (Fig. 26), becomes very thick and is conspicuously stratified (Fig. 27). Furthermore, if the membrane is stretched with needles and then released, it snaps back to its former condition in a manner which reminds one of a rubber band. Finally, when the spores are released from the mature cyst, the latter decreases greatly in size (Figs. 32 and 33), and the membrane becomes very thick. Both Bütschli (1881) and Schellack (1912) noticed the elasticity of the membrane and emphasized its role in discharging the spores, but neither attempted to explain how the sporoducts make their way through the membrane.

The Mature Spore

Shape.—The spores of *Gregarina blattarum* have been described as being barrel-shaped and with truncate ends. Stein (who was the first to describe the spores), Schneider, Bütschli, Marshall, Ellis, Watson, and Schiffmann have given essentially the same description as to the shape. The fact is that, although the spores appear truncate (Fig. 28), they are, in reality, broadly rounded at the ends. The truncate appearance is due to the presence of an external layer of mucoid substance which forms a sheath over the true spore membrane and fills up the space between the rounded ends of the contiguous spores in the chain.

The presence of this sheath is demonstrable in a number of ways. It can be directly observed in optical section (Fig. 29), for the sheath, being less hyaline, appears slightly darker than the true spore membrane. This is particularly evident at the ends of the spore where the sheath is thicker; here it often forms a ring-shaped elevation around the end of the spore. Bütschli called attention to the fact that the terminal portion of the spore covering is darker than the rest, but he did not recognize the significance of this difference. The substance covering the spore dissolves in either dilute or glacial acetic acid. Spores thus treated show well rounded ends (Fig. 30). Finally, when fresh spores are placed under a cover-glass and a slight pressure is exerted on them, the sheath is often removed and is seen as a delicate membrane lying beside the spore, the latter having broadly rounded extremities. The source of the mucoid sheath is probably the viscous mass of mucous substance in which the spores are imbedded during their development.

In cross section the spores appear to be perfectly round, as seen in both fresh and stained preparations. The sides of the mature spore are usually somewhat convex, but not infrequently they appear perfectly parallel to each other. The spore thus varies in shape from an ellipsoid to a cylinder with rounded ends.

Size.—Stein gave the size of the spore as "1/200" lang und 1/570" breit" (which is about $10.8\ \mu$ by $3.8\ \mu$); Ellis gave $4\ \mu$ by $8\ \mu$; Watson "8.3 by 3.7 and 4 by $8\ \mu$." In the present study fifty fresh, mature spores from a single cyst were measured in 0.75 per cent NaCl; and the averages of the long and short axes were found to be $8.77\ \mu$ and $4.30\ \mu$ respectively. These figures are in good agreement with those given by all other workers except Stein, and no explanation of this difference can be offered. It is possible that spores in different cysts differ in size, but this question remains to be investigated.

Structure.—The mature spore consists of the mucoid sheath described above, the true spore membrane, and the contents within. The membrane itself is very hyaline, uniformly rather thick, plainly double contoured,

and with no trace of any surface markings (Fig. 30). Internally, the developing spore appears uniformly structureless except for a large fat droplet which stains dark red with Sudan III. Later, this large droplet breaks up into a variable number of smaller droplets (Fig. 30). At maturity two small refractile spherules, of undetermined nature, are usually seen within the spore, one lying near each end. Frequently faint spiral striations are also seen within the mature spores. The striations at a higher optical level form crosses with those at a lower level. These striations probably represent either the eight sporozoites within or the eight long nuclei of the sporozoites. Attempts to determine their true nature with acidified methyl green and various vital stains were unsuccessful, since the membrane of the spore is very resistant to the penetration of stains.

Spore Chains.—A number of workers have noticed that the spores, as they emerge from the cyst, are associated in long chains; but no one seems to have noted the extremely great length which a single spore chain may attain. The lengths of the chains depend somewhat on the medium in which the expulsion of the spores occurs. In water, relatively short chains are formed; but in air, with the humidity at the saturation point, the chains formed are remarkably long (Fig. 31) and contain many thousands of spores. The idea was conceived that if the chains were straight, it would be easy to measure their lengths. Some cysts were then placed on a glass plate, to which they readily adhered; the excess of water was removed with filter paper; and the plate was inverted over the mouth of a large glass vial containing a small amount of water to maintain a high humidity. When the spores were discharged, they extended directly downward as long straight threads resembling cobweb. Threads as long as 87 mm. and containing approximately ten thousand spores were obtained.

When a large number of cysts from feces are placed in the moist chamber and left to mature, they appear to the unaided eye, on the third day, as if entirely overgrown with fine white threads of mold. These white threads, when highly magnified (Fig. 34), are seen to be chains of the spores. As they pass out of the cyst and come in contact with objects in the vicinity, they often become arranged in large coils, the coils having many turns and thus containing many thousands of individual spores (Fig. 35).

One at once wonders how it is possible for the spores to become associated in such extremely long chains. It is inconceivable that chains of such length are pre-formed within the cyst, although sections of mature cysts show that many of the spores do lie end to end. The probable explanation is that the shorter chains become united as they pass out through the sporoduct. The force exerted by the contracting cyst mem-

brane upon the spores as they pass out presses them together, and the mucous substance covering the spores is sufficiently adhesive to cement them to one another.

When spores discharged into the air are placed in water, they strongly resist wetting and tend to collect on the surface films of air bubbles under the cover glass (Fig. 36). This phenomenon indicates that the spore has a film of oil on the surface. This oil, as was already mentioned, probably serves as a lubricant to aid the spore in passing through the narrow lumen of the sporoduct (Fig. 22).

Nuclear Phenomena

An investigation of the entire nuclear cycle of a gregarine is an enormous task and has been undertaken in only a few instances. In the case of *Gregarina blattarum*, by far the greater part of the previously published observations have concerned the earlier stages. Von Seibold and Leidy made observations on the nucleus of the living sporadin. Bütschli studied the nucleus in the trophic stages and the young cyst, using living material. Wolters studied some of the stages within the young cyst, using sectioned and stained material. Both Marshall and Schiffmann made a number of observations on the nuclear changes throughout the life cycle of this sporozoan. Our knowledge of most of the stages is still quite fragmentary, however, and we have by no means a connected account of the nuclear cycle.

Although it is highly desirable at this time to investigate the entire cycle, technical difficulties involved in preparing good sections of the cysts and a limited amount of time prevent such a study. The present investigation, therefore, is largely confined to those extremely interesting and critical stages which occur in the developing sporoblast and which involve syngamy and the meiotic phenomena. These stages have not yet been worked out in detail for any cephaline gregarine, and conclusions with regard to the time and manner of chromosome reduction in this group have previously been based largely on a priori evidence and analogy. An accurate knowledge of the chromosome cycle requires an understanding of the meiotic division and the stages immediately preceding and following them. Furthermore, a knowledge of these stages probably includes the essential features of a chromosome cycle. It seemed, therefore, most desirable, in view of the fact that meiosis in some of the Acephalina is known to be zygotic, to give particular attention to the chromosome behavior in the developing sporoblast.

Pregametic Divisions and the Gametes.—The pregametic nuclear changes were studied only sufficiently to determine with relative certainty the number of chromosomes involved and the nature of the nuclear divi-

sions. No attempt to give a complete account of the nuclear changes previous to gamete formation is made.

The chromosomes are most easily distinguished in the anaphase. At this time three chromosomes are seen going into either daughter nucleus (Fig. 37). Two are identical in appearance, being very small and almost spherical. The third is considerably elongated. These three chromosomes have a very characteristic arrangement in the anaphase; the two small ones lie side by side and migrate in advance, while the third trails behind. The latter has frequently been called the "odd chromosome," since it differs in appearance from the other two. This designation is, however, inappropriate, as Jameson has pointed out, for it implies that the other two chromosomes constitute a pair. The fact is that if subsequent events are correctly interpreted, all three of the chromosomes are "odd" in the sense that none of them is paired. Very frequently the long chromosome appears to be more closely associated with one of the small ones than with the other, but this relationship is not always evident.

For the sake of convenience, the chromosomes may be numbered on the basis of their appearance in the anaphase. Throughout the following description the long chromosome will be designated as number 3, the short one nearest to it will be called number 2, and the other short one number 1. It is true that the basis for distinguishing 1 and 2 is very flimsy, for the spatial relationship of these two to 3 may not be constant and is certainly not always apparent. But since some method of designation is almost indispensable for purposes of description, the only one available at the present time is used.

The anaphase chromosomes move farther apart (Fig. 38) and merge into a small amorphous granule in the telophase (Fig. 39). This granule transforms itself into a vesicular nucleus (Fig. 40) from which the prophase chromosomes of another division emerge (Fig. 41). Finally, after an undetermined number of divisions, these nuclei become the nuclei of young gametes (Fig. 42) which bud off from the periphery of the former gamont.

The gamete, at the time it separates from the parent mass, is a small pyriform body, about $3\ \mu$ by $1.5\ \mu$, with reticulated cytoplasm and a vesicular nucleus near the anterior end (Fig. 43). Very frequently the nucleus appears to be nothing but a small crescent-shaped chromatic granule lying with its convex side toward the anterior end of the gamete. On the other hand, it often appears to be a small vesicle surrounded by a delicate nuclear membrane, with most of the chromatin collected into a large mass on one side. Schiffmann thought that most of the chromatin collects on one side of the nucleus and is then thrown out, the process constituting a "primitive Reifeteilung." Nothing resembling this process

was seen during the present study. Nor was extranuclear chromatin seen in the gamete, although, according to Jameson, a number of workers have reported extrusion of a chromatin granule from the nucleus of the gamete in various species of *Gregarina*.

The newly formed gametes round up almost immediately, and both the nucleus and cytosome begin to increase in size. Growth continues until the original diameter of the gamete has increased by about one-half. During the increase in size, and probably correlated with it, a large vesicle appears around the nucleus (Fig. 44). This perinuclear vesicle is probably bounded by a delicate membrane. Both the nucleus and perinuclear vesicle are eccentrically located in the gamete, and the nucleus lies on the outer side of the vesicle with its chromatin granule directed anteriorly. Sometimes it seems that very faint threads can be seen radiating out from the chromatin granule into the karyolymph. These may actually represent the chromonemata of the future chromosomes. And, as later events indicate, the chromatin granule itself is probably a reservoir of nucleic acid which supplies the chromophilic component of the chromosome matrix. All the gametes appear to be morphologically alike, although a detailed cytological study might reveal differences in the nuclei or in the cytoplasmic components. Behavior during syngamy, however, suggests physiological differences in the copulating gametes.

Syngamy, Synapsis, and the First Metagamic Division.—At the beginning of syngamy two gametes come together in such a manner that a point slightly to one side of the nucleus on one individual comes in contact with another individual at an exactly opposite point on the other side. The posterior gamete, which is the more active one, may be designated, for the sake of convenience, as male and the other female. Cytoplasmic fusion of the two gametes then follows (Fig. 45). Soon the two perinuclear vesicles fuse into one, and the male pronucleus moves up toward the female one while the latter remains stationary (Fig. 46). The two pronuclei thereby come to lie in a common perinuclear vesicle, the membrane of which serves as the nuclear membrane of the future synkaryon.

During or at the end of the period of migration of the male pronucleus, its membrane breaks down and an unraveling of its three chromosomes occurs (Fig. 47). In some cysts the male pronuclei seem to behave quite differently. In such cases the individual chromosomes can rarely be distinguished (Fig. 48), but are typically associated together in the form of a more or less complete loop posterior to the female pronucleus (Fig. 49). This type of behavior could not be reconciled with that described above and may indicate that two different varieties of *Gregarina blattarum* are involved.

Immediately following or overlapping the change in the male pro-

nucleus, three short and coiled chromosomes become distinguishable in the female pronucleus; the membrane of the latter breaks down (Fig. 50); and three relatively long leptotene threads emerge (Fig. 51). It is thus evident that a complement of three chromosomes is contributed to the synkaryon (zygote or sporoblast nucleus) by each of the two copulating gametes. The haploid number is, therefore, three, and the diploid number is six.

The three chromosomes of each haploid complement are typically close together at one end (Fig. 51). Then the two complements come together in a polarized arrangement resembling that which is, according to Wilson (1925), common in the early prophase of the first meiotic division in animals (Fig. 52). The polarized chromosomes typically lie in the same position as that occupied by the female pronucleus at the beginning of syngamy, for that nucleus never changes its orientation throughout the process. In other words, the six chromosomes are so polarized that, if seen from a side view, all are directed toward such a point on the anterior end of the zygote as to form an angle of about forty-five degrees with the long axis.

At about the time the leptotene threads attain their maximum length they arrange themselves into pairs which are polarized as before (Fig. 53). This pairing probably is a true synapsis involving the association of homologous chromosomes of maternal and paternal origin. The two synaptic mates in a given pair look identical, and corresponding chromomeres can frequently be distinguished on them (Figs. 53 and 54). Further study of the chromomeres may possibly enable one to identify each of the three chromosomes at this stage.

The zygonemata enter into a typical pachytene stage by becoming shorter and thicker (Fig. 54). One pair, in the pachytene stage, is characteristically much thicker and smoother in outline than the other two and lies close to the nuclear membrane near the anterior end of the zygote. The pair which thus appears different from the other two probably represents chromosome number 3, although this point has not definitely been established.

A diplotene stage was not distinguished, although each synaptic mate has presumably become double by a longitudinal splitting by about this time.

The chromosomes continue to become shorter and thicker, and lose their polarization (Fig. 55); the definitive tetrads become arranged around the periphery of the nucleus (Fig. 56). At this stage each tetrad appears to consist of two parts, but a quadripartite composition is assumed because of the interpretation which the author places upon subsequent observation. The assumption is admittedly based, in part, on analogy with the condition known to exist in many of the metazoa. The

author can only point to the difficulties encountered in studying the relatively large chromosomes of the metazoa, and claim justification for frequently being unable to observe directly the actual conditions in structures which approach in smallness the limits of visibility.

The next change is a precocious disjunction in tetrads 1 and 2, so that four dyads and one tetrad (number 3) are seen near the periphery of the nucleus before the nuclear membrane breaks down (Figs. 57 and 58). The tetrad is not always distinguishable; but when it is properly oriented, it appears V-shaped (Fig. 58).

After the breakdown of the nuclear membrane the chromosomes usually lie in a compact clump, so that the individual chromosomes are rarely distinguishable (Fig. 59). Occasionally it can be seen that about this time the chromosome complex consists of a V-shaped tetrad (number 3) and two groups, each containing (probably) dyads 1 and 2 lying side by side (Fig. 60). A "typical" equatorial plate does not occur, because disjunction of two of the three tetrads takes place before the metaphase. No trace of an achromatic figure was seen at this or any other stage.

In the early anaphase (Fig. 61) dyads 1 and 2 of one set remain stationary, and those of the other set begin to migrate along an axis diagonal to the long axis of the sporoblast. The V-shaped tetrad (number 3) lies between the two groups of dyads. The dyads continue to migrate, and the two arms of tetrad number 3 move apart (Fig. 62).

During the anaphase a diamond-shaped clear space frequently appears in the region where dyads number 3 are still joined at one end (Fig. 63). Delayed disjunction in this region probably results in a force of repulsion along the equatorial plane, making all four chromatids distinguishable. At no other stage is the bivalent nature of the dyads apparent.

As the anaphase progresses, the direction of migration, which was at first diagonal, changes so that the two groups of dyads come to lie exactly opposite one another in the two ends of the sporoblast. Meanwhile, a very fine but distinct and dark-staining thread is frequently seen extending between the two number 3 dyads as they move apart (Figs. 64 and 65). In the anaphase, when the three chromosomes are most easily distinguished, they are obviously much larger in the first metagamic division than in any other division. This is due, very likely, to their bivalent composition.

In the telophase a vesicular nucleus is reconstructed with the bivalent chromosomes lying on the periphery (Figs. 66 and 67). The individual chromosomes then become indistinguishable, and the interphase condition results (Fig. 68). The nucleus in the interphase is vesicular, faintly reticulated, and has a number of small chromatin granules scattered

around the periphery. Quite a complete reorganization is thus seen to occur at the end of the first division, resulting in a relatively long interphase.

It is evident from the foregoing account that the first metagamic division is a meiotic division. The zygote (sporoblast) nucleus is diploid, each gamete having contributed a haploid set. Synapsis of homologous chromosomes occurs and is followed by tetrad formation and disjunction. Each daughter nucleus resulting from the first metagamic division contains the haploid number (three) of bivalent chromosomes.

Second Metagamic Division.—In the prophase of the second division the chromosomes emerge from the interphase as relatively long threads lying on the periphery of the nucleus (Fig. 69). The number present at this time is difficult to determine but is sufficiently large to demonstrate that the bivalents have already separated into univalents, except number 3 in which separation occurs in the anaphase. The probable condition is that four univalents and one bivalent are present. Sharp (1934) says: "In the prophase of the second meiotic division the condensing chromosomes appear characteristically in the form of threads or rods, still associated in dyads at their attachment regions but diverging widely elsewhere." Since no spindle fibers and therefore no attachment regions seem to occur here, the chromatids, as one would expect, seem to separate completely in the prophase.

A "typical" metaphase does not seem to occur. The nuclear membrane breaks down long before the chromosomes have reached their definitive form, and the chromatin threads become clumped together in a confused mass (Figs. 70 and 71). This stage resembles the metaphase of the first division but is much more confused, and the chromosomes are relatively longer and thinner. The long threads then condense and assume their final form (Fig. 72), but it is difficult to distinguish any orderly arrangement before the anaphase.

The early anaphase in the second division (Fig. 73) resembles very closely that in the first except that the chromosomes, being now univalents, are smaller. The direction of migration is diagonal as before; chromosomes 1 and 2, lying side by side, move ahead while 3 trails behind. The chromosomes do not come together in such a way that one end of each meets an end of the other two (see Fig. 66); but they arrange themselves in the form of an angle, number 2 being at the corner and 1 and 3 forming the sides (Fig. 74).

In the telophase the three chromosomes fuse (Fig. 75); the angle becomes bent into a half circle and finally forms a complete circle (Fig. 76). Immediately upon the formation of the complete circle, the nucleus is transformed into a sphere. The resulting interphase nucleus (Fig. 77)

becomes a vesicle with chromatin granules scattered on a faint reticulum. No explanation as to the manner in which the circular nucleus becomes transformed into a vesicular one can be offered at this time.

The termination of the second metagamic division marks the end of the meiotic phenomena. The zygote is a meiocyte; and the first division involves synapsis, tetrad formation, and disjunction. In the second division the two chromatids in each dyad separate; and to each of the resulting four nuclei a haploid complement of univalent chromosomes, like that in the gamete, is restored.

Throughout the preceding description of the meiotic divisions it has been assumed, for the sake of convenience, that the first division is disjunctional and the second equational. Actually, the reverse condition may hold true; or it may be that each of the divisions is disjunctional for some elements of the complex and equational for others. Sharp states: "The four chromatids composing a tetrad are ordinarily similar to one another in appearance when fully condensed, so that it is difficult or impossible to determine by direct observation whether the separation in the first anaphase is along the synaptic or along the equational plane Furthermore, since the several tetrads in one mitotic figure may behave differently in this respect, each of the two mitoses in such cases may be disjunctional for some elements and equational for others. Taking the chromosome complement as a whole, the disjunction of its homologous elements and the equational separation of their halves are complete only at the conclusion of the second mitosis."

Third Metagamic Division and Subsequent Nuclear Changes.—The prophase chromosomes of the third metagamic division emerge from the preceding interphase as three polarized strands (Fig. 78). It is difficult to understand how this arrangement is possible, since the chromosomes went into the previous telophase in the form of a ring; but the answer probably depends on an explanation of the process involved in the transformation of the telophase ring into the interphase vesicle. No explanation of these phenomena is suggested. The nuclei are considerably smaller than in the two previous divisions and little detail can be distinguished. In the later prophase the chromosomes are usually seen as three relatively long threads all lying parallel to one another (Figs. 79 and 80).

The metaphase resembles that in previous divisions. After the breakdown of the nuclear membrane (at an undetermined time), the chromosomes lie in a confused clump (Fig. 81). They are not individually distinguishable until the anaphase begins.

In the anaphase four groups of small chromosomes (representing the four dividing nuclei) are seen, each with the characteristic arrangement seen in earlier nuclear divisions. Chromosomes 1 and 2 migrate ahead in an oblique direction while 3 trails behind (Figs. 82 to 84). The late

anaphase is like that in the second, the chromosomes being so arranged as to form an angle.

The three chromosomes in each set become fused in the early telophase to form a bent rod (Fig. 85). The rod becomes further bent to form an incomplete circle (Fig. 86), and then the two ends come together and make a complete circle (Figs. 87 and 88). The circles lie in two groups of four each near the two ends of the spore. Up to this point the third telophase resembles the second; but here the resemblance stops, for the nuclear ring does not become transformed into a vesicle.

As the telophase reorganization continues, two conspicuous granules appear on each ring (Fig. 89). The two granules are on opposite sides of the ring, and the latter is oriented in such a way that a line passing through the two granules is parallel with the long axis of the spore. Usually one granule is plainly larger than the other. This is particularly evident when the nuclear ring lies on edge (Figs. 88 and 89). The smaller of the two granules is directed toward the end of the spore in some cysts, although the reverse orientation occurs in the spores of other cysts and is described later. Judging from the appearance of the nucleus during the telophase reorganization, it seems probable that the larger granule represents the bulk of the chromatic substance of chromosomes 1 and 2 combined, while the smaller represents that of chromosome 3.

The next change is an elongation of the nuclear rings. Each transforms itself into a long oval with a chromatin granule at either end (Fig. 90). The ovals, in a manner not clear, then change into dumbbell-shaped rods which are usually plainly larger at the inner end than at the outer end (Fig. 91). Finally, the rods become further elongated and come to lie in a spiral arrangement around the peripheral region of the spore contents (Fig. 92). Since all the nuclei are spirally arranged in the same direction, those in a higher optical plane typically lie across those in a lower optical plane. Previous workers seem to have overlooked the elongated condition of the sporozoite nucleus in *Gregarina blattarum*, although Prowazek (1902) has observed a similar shape and arrangement of the nuclei in the spore of *Monocystis agilis* Stein.

No further normal nuclear changes were observed, but in mature cysts the great majority of the spores contain degenerating nuclei. The chromatin in these degenerate spores is seen in scattered masses inconstant in size, number, and arrangement. This fact may indicate that the spores remain viable for only a very short time after maturity, though experimental data on the viability of the spores is lacking. Many of the mature spores show no trace of any internal structure by any of the methods used. This may be due, at least in part, to the inability of the stains to penetrate the resistant spore membrane.

At no time were the cytoplasmic boundaries of the individual sporo-

zoites observed with certainty, although each spore is believed to contain eight sporozoites. As stated above, mature spores in the living condition frequently show faint spiral striations. Whether this appearance is due to the sporozoites or to the eight long spirally arranged nuclei is uncertain.

The nuclear phenomena in the final stage are in some instances quite different from those described above. In some cysts all the eight nuclei in every spore lie at or near the center of the spore. In such cases the larger chromatin granule is directed not toward the center but toward the end of the spore (Fig. 93). Elongation occurs with apparently little or no change in the location of the nucleus (Figs. 94 and 95). It should be emphasized that all the spores in one cyst are of one type with regard to the location and orientation of the nuclei. The different conditions observed do not seem to fit into a single pattern of development. This and other discrepancies observed suggest that two or more types of *Gregarina blattarum* may exist.

DISCUSSION OF CHROMOSOME BEHAVIOR IN GREGARINES

JAMESON (1920) and Naville (1931) have given complete historical accounts of the chromosome cycle in gregarines with critical discussions of the various phases. It would be superfluous to review at this time the same material in detail; but certain aspects of the chromosome cycle, upon which the present investigation has a direct bearing, may be profitably considered.

TYPES OF REDUCTION

Cephalina

According to Naville (1931), "L'étude du cycle chromosomique, des phénomènes réductionnels et de la fécondation des *Cephalina* est beaucoup moins avancée que celle des *Acephalina*. Pour aucune espèce nous ne possédons jusqu'à ce jour d'étude complète du cycle chromosomique telle qu'il en a été publié pour *Monocystis* par Mulsow et Naville, pour *Diplocystis* par Jameson et pour *Urospora* par Naville . . . Chez les *Polycystidés* nous ne possédons que quelques documents fragmentaires."

Naville believes that the lack of information on the *Cephalina* is probably due to the difficulty of collecting the cysts in very great numbers, since they are expelled from the host soon after formation. By the methods used in the present studies, the difficulty of obtaining cysts of *Gregarina blattarum* in unlimited numbers, and at any desired stage of development, has been overcome; and the author believes that he has been able to demonstrate the details of meiosis for the first time in a

cephaline gregarine. Chakravarty (1935) recently made a study of *Hyalosporina cambolopsisae* Chakravarty, a cephaline form. Regarding meiosis in that gregarine he stated, "It is quite evident from the study of sections that the number of chromosomes is two and that the first division of the zygote nucleus is the meiotic division." Although the conclusion of Chakravarty may be justified on the basis of what he saw, his published observations and figures do not supply enough information for one to judge the correctness of the statement.

Early workers attempting to discover meiosis in cephaline gregarines have, as Jameson pointed out, concentrated their attention on the pregametic divisions. Several of them have clearly demonstrated an odd number of chromosomes in those stages. This is good a priori evidence of zygotic reduction; but clear and detailed descriptions of the process, based on actual observation, seem to be lacking. The author, therefore, agrees with Naville (1931) who stated that it is impossible at the present time to decide whether zygomeiosis is the rule in the Cephalina.

Acephalina

The chromosome cycle has been studied much more successfully in the Acephalina, and both gametic and zygotic meiosis are known to occur. Gametic meiosis was described in Monocystis by Mulsow (1911), Bastin (1919), Naville (1927a), and Calkins and Bowling (1926), and has been described in Urospora by Naville (1927). Zygotic meiosis was described in Diplocystis by Jameson (1920) and in Zygosoma by Noble (1938).

The work of Noble on Zygosoma, being perhaps the most conspicuous piece of work published on the chromosome cycle in gregarines since Naville's (1931) comprehensive review of the subject, requires particular attention here. In spite of Noble's unquestionably conscientious work, a careful study of his paper leads the present writer to feel that the material upon which Noble based his "sequence of changes immediately preceding and following the formation of the zygote" (most of the essential stages of the chromosome cycle) may have been in an advanced state of degeneration. The following observations are made in support of this view:

(1) "Out of approximately 150 cysts kept under various conditions in the laboratory, only one developed as far as the sporoblast stage." Apparently no other cyst in a late stage of development was observed. The wisdom of basing a number of important conclusions on one specimen in any case requires no comment, but when only one out of 150 cysts is not obviously in a stage of disintegration, that one certainly should arouse suspicion, and conclusions based on it cannot be very convincing. It is noted that Noble, himself, expressed some uncertainty as to whether that one cyst was entirely normal.

(2) The cyst studied contained all stages from gametes to spores with four nuclei. In normal cysts of *Gregarina blattarum* the rate of development is practically uniform throughout the cyst; obviously degenerate specimens often show an unequal rate of development. Although other workers have not been very specific on this point, one gains the impression that an essentially uniform rate of development within the cyst is common in gregarines.

(3) The nuclei of gametes, zygotes, and spores in many of Noble's figures are irregular masses of chromatin which have more the appearance of degenerating nuclei than of normal ones.

(4) Both the nucleus and cytoplasm in many of the stages represented are highly vacuolated. It is well known that vacuolization frequently accompanies degeneration.

(5) Noble observed, "The zygote nucleus does not divide until after the sporocyst membrane is fully formed." This is unusual; it suggests that the nucleus may have become inactive due to some abnormal condition and that the membrane simply continued its development for a time.

(6) The nucleus of the zygote "becomes dumbbell-shaped, and pinches in two." This behavior is very suggestive of a state of degeneration; for it seems inconceivable that a division which is regarded as meiotic can, at the same time, be amitotic.

(7) The nuclei of developing sporoblasts, when not vacuolated, are compact. This appearance was noted by the present writer in degenerating nuclei of *Gregarina blattarum* in which the normal nucleus is vesicular, ring-shaped or rod-shaped, depending on the stage.

(8) The spores were found to have only four nuclei rather than eight, which is common in gregarines. The most reasonable explanation is that development simply did not proceed any further.

(9) Some spores, which the author admitted to be abnormal, contained five nuclei rather than four. This point is highly significant in the light of experience with *Gregarina blattarum*, in which it was found that all the spores in a given cyst either develop in a normal manner or all degenerate. The presence of some admittedly abnormal spores in a cyst throws suspicion on other spores within the same cyst.

(10) The second metagametic division, like the first, appears to be by constriction. Here, again, the type of division seems to suggest an abnormal condition of the nucleus.

(11) Although none of these criticisms alone may be conclusive, all of them taken together seem to justify the belief that a renewed study of *Zygosoma globosum*, using more abundant material, might yield significantly different results.

In summarizing the types of reduction found in gregarines, it may be said that both zygotic and gametic meiosis are known to occur in the

Acephalina; and now direct and detailed evidence is produced to support the a priori belief that zygotic reduction occurs in some, at least, of the Cephalina.

SYNOPSIS

In general, our knowledge of synopsis in gregarines is quite fragmentary. This may be due, at least in part, to the fact that synopsis seems to be common in the zygote; and early workers neglected this stage in their studies. More recent investigations have given us some definite information concerning both the haploid and the diploid forms.

Haploid Forms

Schellack (1912) mentioned synopsis in the zygote of *Gregarina ovata*, which is probably a haploid form, and apparently indicated the process in his figures, though he gave no detailed description. The process, as judged by the figures given, strikingly resembles that in *Gregarina blattarum*, since the chromosomes assume a polarized arrangement before karyogamy and maintain a similar arrangement during synopsis. A more detailed study of the chromosome behavior in the developing sporoblast of *Gregarina ovata* is much desired.

Jameson mentioned synopsis in *Diplocystis schneideri*; but the time at which it is said to occur seems rather unusual, as the following step by step study of the first metagamic division suggests. In the early stages of karyogamy, according to Jameson, "the two little clumps of chromatin granules are at first separate. . . ., but they later unite to form a large mulberry-like karyosome which contracts subsequently to form a more compact body. . . ." This union of the two chromosome complements, immediately after fusion of the nuclei, and the subsequent "contraction" were not regarded as synopsis, although the present author notes that they occupy the same order in the sequence of changes as the phenomenon of synopsis in *Gregarina blattarum* and other haploid gregarines on which we have any information. Jameson further stated: "The karyosome commences to break up. Round particles are given off from it, which seem to move outwards along the achromatic strands towards the periphery of the nucleus." It is observed here that this process of outward movement corresponds, in the time sequence, to disjunction and the outward movement of the dyads in *Gregarina blattarum*. The next nuclear change in *Diplocystis schneideri* is a second contraction. Then the knot opens out. Jameson stated: "As the tangle becomes less obscure one can see that the spireme during this synopsis has become divided into six chromosomes." It was thus this second "contraction" which Jameson regarded as a process of synopsis. The present author observes that this "synopsis," being followed immediately by the anaphasic move-

ment, occupies the same position in the series of changes as the metaphase of ordinary mitosis and the "confused clump" metaphase in *Gregarina blattarum*. A careful and detailed comparison of the sequence of nuclear changes in the zygote of *Diplocystis schneideri* with that in *Gregarina blattarum* and with that in the auxocyte in metazoa thus leads the present author to consider that the first "contraction" described by Jameson may be, in reality, a process of synapsis, while the second "contraction" probably represents the metaphase.

Further information on synapsis has been given by Noble, who has reported that in *Zygosoma globosum* "a process of synapsis immediately follows fertilization." This, he believes, "is the first reported instance of a definite synapsis in a nonseptate gregarine with zygotic reduction."

Finally, in *Gregarina blattarum* the two sets of chromosomes unite to form three pairs immediately after the union of the two pronuclei. The zygonemata have a polarized arrangement resembling that which is common in the auxocyte of metazoa. More information is thus added to our meager knowledge of synapsis in the haploid gregarines.

Diploid Forms

Naville (1927) described in detail, and figured very clearly, synapsis in *Urospora lagidis*. This, he remarked, "n'avait point été décrite jusqu'à ce jour chez les Gregariniens." The zygote nucleus is at first vesicular with peripheral chromatin and an endosome, no chromosomes being recognized. Next there is a preleptotene stage, from which the chromosomes emerge in pairs. The paired condition is of rather long duration and resembles synapsis in the auxocytes of metazoa. A slight difference is noted here between *Urospora lagidis* and *Gregarina blattarum*. In the latter the preleptotene changes occur in the pronuclei; and at the time of karyogamy the leptotene threads are fully formed and ready to go into synapsis. Thus, there is no stage in the synkaryon during which the chromosomes are not recognizable.

In three species of *Monocystis*, Naville (1927a) did not find any conclusive evidence of synapsis but supposed, by analogy with *Urospora*, that the process probably follows immediately after fertilization. The same author (1931) has also pointed out that the figures of Calkins and Bowling (1926), representing the zygote of a species of *Monocystis*, show the chromosomes rather definitely arranged in pairs.

The fact of particular interest with regard to synapsis in both the haploid and the diploid gregarines is that this process always occurs, at least in well known cases, in the nucleus of the zygote. Reduction in the chromosome number, on the other hand, follows immediately after synapsis in the zygote of the haploid forms (*Gregarina*, *Diplocystis*,

Zygosome), while it is delayed until the process of gamete formation in the diploid gregarines (Urospora, Monocystis). Naville (1927, 1931) made the fascinating observation that the diploid gregarines, in which reduction alone is delayed, represent a condition intermediate between the haploid gregarines, which have both synapsis and reduction in the zygote, and the metazoa in which both processes are delayed until the time of gametogenesis.

These three types of chromosome cycles may be represented in tabular form as follows:

	Zygote		Soma		Gamete	
Haploid gregarines . . .	2N	synapsis, meiosis	N		N	syngamy
Diploid gregarines . . .	2N	synapsis	2N	meiosis	N	syngamy
Metazoa	2N		2N	synapsis, meiosis	N	syngamy

Naville (1931) has expressed the entirely reasonable opinion that those forms with both synapsis and reduction in the zygote (Cephalina, certain Acephalina, Schizogregarinaria) represent a primitive condition, while the long delayed reduction in certain of the Acephalina is a more recent acquisition. If this opinion of Naville is correct, the implications with regard to the evolution of sex are obviously rather far-reaching. Possibly further study of the chromosome behavior in gregarines may contribute much more to our knowledge of the evolution of sex in the animal kingdom. Furthermore, if this difference in chromosome cycle is valid and fundamental, it may be a more natural basis for distinguishing the major groups of the gregarines than the presence or absence of a septum, the latter being merely a recent acquisition in certain haploid forms.

NUMBER OF MEIOTIC DIVISIONS

Haploid Forms

Previous workers have given us but little direct information concerning the number of divisions involved in the meiotic process in the haploid gregarines. Since, in the known forms, both synapsis and reduction are zygotic, one must admit that on theoretical grounds two mitoses may be necessary, if tetrads are formed, to accomplish both the disjunction of the homologous elements and the equational separation of their halves. Each of the two mitoses may in such cases, to use the words of Sharp, "be disjunctional for some elements and equational for others."

Schellack apparently observed synapsis and tetrad formation in *Gregarina ovata*. He did not, unfortunately, study subsequent changes. Further investigation of this form is highly desirable; but apparently, as Naville stated, two divisions are necessary to complete the reduction.

Jameson, who has given the most detailed description of the meta-

gamic divisions in a gregarine of this type, demonstrated numerical reduction in the first division and assumed the process of meiosis to be complete at that stage. Since synapsis and tetrad formation were not described, and since the second and third divisions were described very briefly, it is impossible to judge whether the second division is, in any sense, a meiotic division. Jameson, himself, was noncommittal on this question; but he gave no evidence to exclude the possibility, which theoretically exists, that meiosis may not be completed until the end of the second division. In *Zygosoma globosum*, Noble likewise postulated a numerical reduction in the chromosomes in the first metagamic division and gave the question no further consideration.

In the present paper, for the first time in gregarines, two meiotic divisions are described in *Gregarina blattarum*. Aside from theoretical considerations, certain definite, though admittedly inconclusive, observations seem to support the belief that the first two metagamic divisions should both be regarded as meiotic. Both synapsis and meiosis are zygotic, and tetrads seem to be formed in the prophase of the first division. If tetrads actually are formed, then two meiotic divisions may be a necessity. Chromosome number 3, during the first anaphase, frequently shows evidence that splitting has occurred at an early stage in the first division; this suggests that the first mitosis may be disjunctional for some elements and equational for others, in which case a second meiotic division is required to complete the separation of the maternal and the paternal elements. The chromosomes emerging from the first metaphase are larger than any seen elsewhere (except the tetrads); this is presumptive evidence of a bivalent nature and a further indication that equational splitting of the homologous chromosomes may occur in the early part of the first division. The prophase of the second division is characteristic of a second meiotic division, since the elements of the dyads become separated in that stage. In conclusion, it may be said that the series of nuclear changes in the first and second metagamic divisions of *Gregarina blattarum* appear strikingly similar, in all essential respects, to the meiotic divisions in metazoa. Further study on this and other haploid gregarines is necessary to confirm the apparent similarity; for, since the chromosomes are exceedingly small, certain of the questions involved can be positively answered only with the greatest difficulty.

Diploid Forms

In those gregarines with zygotic synapsis and gametic meiosis there is no apparent reason to suppose that more than one nuclear division is needed to complete the process of reduction; yet in some of these forms, at least, that division which accomplishes numerical reduction seems to

be followed by one or more equational divisions. Mulsow (1911) could not decide whether numerical reduction is followed by another division in *Monocystis*, while Calkins and Bowling thought that reduction occurs in the last pregametic division. Bastin (1919), on the other hand, believed that in *Monocystis agilis* Hesse the reduction is accomplished in two steps, as in the metazoa, although he was unable to observe the details. It is important to note that Bastin searched in vain for synapsis previous to the reduction division.

Naville (1927) seems to have clearly demonstrated a period of division following numerical reduction in *Urospora lagidis* (de Saint Joseph). The same process was found by Naville (1927a) in three species of *Monocystis*. He (1931) believed that Mulsow and also Calkins and Bowling overlooked the last period of division. Thus, the occurrence of one or more mitoses following numerical reduction in the chromosomes may be the rule in the diploid gregarines. There is, however, no convincing evidence that these mitoses are involved in the meiotic process.

GENERAL CONSIDERATIONS

At this time certain general remarks may be made with regard to the chromosome cycle in gregarines:

(1) Since in the diploid forms meiosis is gametic and not immediately preceded by synapsis (the latter being zygotic in known instances), a single meiotic mitosis is sufficient to accomplish both numerical reduction of the chromosomes and the separation of the maternal and paternal elements.

(2) Any division following numerical reduction in these forms cannot, therefore, be regarded as being, in any sense, meiotic.

(3) In the haploid gregarines, on the other hand, synapsis and meiosis are both zygotic (insofar as we are able to judge at the present time). One must admit, therefore, that two mitoses may be necessary to accomplish both the disjunction of the homologous elements of the chromosome complement and the equational separation of their halves.

(4) Among previous workers only Jameson has given us anything approximating an adequate description of the second metagametic division in haploid gregarines; and he disregarded its possible role in meiosis.

(5) In *Gregarina blattarum* the metagametic divisions have been worked out in detail for the first time in a cephaline gregarine. The series of nuclear changes following syngamy appears to be similar, in all essential respects, to that found in maturation in metazoa. Detailed studies on other haploid forms are needed to confirm the opinion that meiosis is accomplished not in one division but in two, as in the metazoa.

(6) Finally, it is highly desirable that some method be devised for

identifying the chromosomes in the various stages, so that they can be individually followed through the cycle. A number of questions could thereby be positively answered. One might, for instance, determine definitely whether the two small chromosomes seen in the haploid nucleus in some gregarines actually belong to the same pair or to different pairs. This must still be regarded as an unanswered question and one of fundamental importance with regard to the pattern of the chromosome cycle. A dependable method of identifying the three chromosomes of *Gregarina blattarum* at all stages might lead, also, to an understanding of the many differences in the behavior of the short chromosomes and the long one. Further, the pattern of the chromosome cycle might be altogether different from that postulated in the present study or that known elsewhere among animals. At present, one must, admittedly, interpret the observed phenomena in a manner which seems most plausible in view of the facts known about chromosome behavior in other animals. One must, at the same time, admit that more precise data might lead to fundamentally different conclusions.

SUMMARY AND CONCLUSIONS

DURING the fall and winter of 1939 (September to the middle of December) 810 specimens of *Blatta orientalis* were examined for *Gregarina blattarum*. Only 5.3 per cent were found to be infected, and in each case the infection was light. At that time cysts were seen but rarely.

In January and February of 1940 about 30 per cent of a collection of roaches, which had been kept in the laboratory since the previous fall, were very heavily infected. Some of the cysts were fed to the roaches in an attempt to maintain a high incidence of infection, and others were used in these studies.

The process of encystment was observed a number of times after pairs of mature gamonts were placed in fresh undiluted egg albumen on a depression slide. The observations made on this process are in essential agreement with those made by Bütschli, but an attempt is made to explain why the newly formed cyst changes from a sphere to a prolate spheroid. The average ratio of the long to the short axis of fourteen cysts formed on a slide was found to be 1.76. Of 25 cysts formed in the same host from which the other material was obtained the average ratio was 1.22. These results indicate that the amount of restriction as to space during a certain critical period of encystment is an important factor in determining the shape of the cyst.

A scatter diagram shows a positive correlation between the long and short axes of a random sample of cysts indicating little or no tendency for the shape to vary with the size.

A histogram showing size distribution of cysts indicates that encystment seldom occurs until the gamonts have attained a certain definite minimum size.

The externally visible features of cyst development are described, and an explanation of sporoduct eversion is proposed. Neutral red and bromthymol blue indicate an acid reaction in the basal region of the sporoduct. Enzymes acting in an acid medium probably have a lytic effect on that portion of the cyst membrane which is in contact with the basal disc of the sporoduct. The membrane becomes weakened at that point, and the great pressure exerted on the cyst contents by the elastic membrane forces the sporoduct through the weakened area in the membrane.

Cysts kept until maturity in a moist chamber discharge their spores in very long chains. One continuous chain may contain as many as 10,000 spores. The chains of spores often become arranged in large coils containing many turns.

The mature spore is not truncate as described by previous workers; but it is covered by a mucoid sheath which gives it a truncate appearance. The sheath is adhesive and holds the spores together in the chain.

A detailed description of the nuclear changes during spore development is given. In the pregametic divisions three small chromosomes with a characteristic appearance are plainly seen. They are designated as numbers 1, 2, and 3.

The characteristic position assumed by the two gametes during syngamy and differences in the behavior of the pronuclei suggest physiological differences in the two copulating gametes. One of them is, therefore, designated as male and the other female.

A haploid set of three chromosomes is contributed to the synkaryon by each of the two pronuclei. The six chromosomes assume a characteristic polarized arrangement and undergo synapsis and tetrad formation. In the anaphase of the first metagamic division three dyads go to each daughter nucleus. The two elements of the dyads are then separated in the second metagamic division, resulting in four nuclei, each with a haploid complement of three chromosomes. It is thus seen that the zygote is the only diploid stage, all others being haploid.

The third metagamic division results in eight ring-shaped nuclei which transform themselves into long rods. These are the nuclei of the eight sporozoites.

Certain variations in the size of the cysts and discrepancies in the nuclear phenomena suggest that two or more varieties of *Gregarina blattarum* may be involved in these studies.

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PLATES

ALL DRAWINGS were made with the aid of an Abbe camera lucida. Figs. 1-20 were drawn from living material and are magnified approximately 60 times. Figs. 21-36 represent various methods and magnifications as indicated. Figs. 37-95 are from smear preparations, fixed and stained by methods indicated; they are magnified approximately 1850 times. Abbreviations are as follows: *S.*, Schaudinn; *Z.*, Zenker; *H.*, Heidenhain; *F.*, Feulgen; *G.*, Giemsa.

PLATE I

- FIG. 1.—An unusually large sporadin; the only individual present in the host.
- FIG. 2.—A trophozoite with epimerite and three pairs of very small gamonts in syzygy. The latter show that pairing may occur in very immature individuals.
- FIG. 3.—A rather rare instance of multiple association.
- FIGS. 4-11.—Successive stages in the process of encystment, as seen in one pair.
- FIG. 4.—Beginning of encystment.
- FIG. 5.—About 15 minutes later. Opposite ends are brought together.
- FIG. 6.—About 10 minutes later. The two individuals are more closely associated.
- FIG. 7.—About 5 minutes later. The two individuals appear to be firmly cemented together.
- FIG. 8.—About 15 minutes later. A cyst membrane is being secreted; the young cyst is almost a perfect sphere.
- FIG. 9.—About 15 minutes later. The cyst rather suddenly begins to elongate.
- FIG. 10.—About 15 minutes later. The cyst is still in the process of elongation and the membrane is thicker.
- FIG. 11.—About 1 hour later. The cyst is complete; but the membrane is abnormally thick at the ends, since rotation of the individuals within is inhibited by excessive elongation.

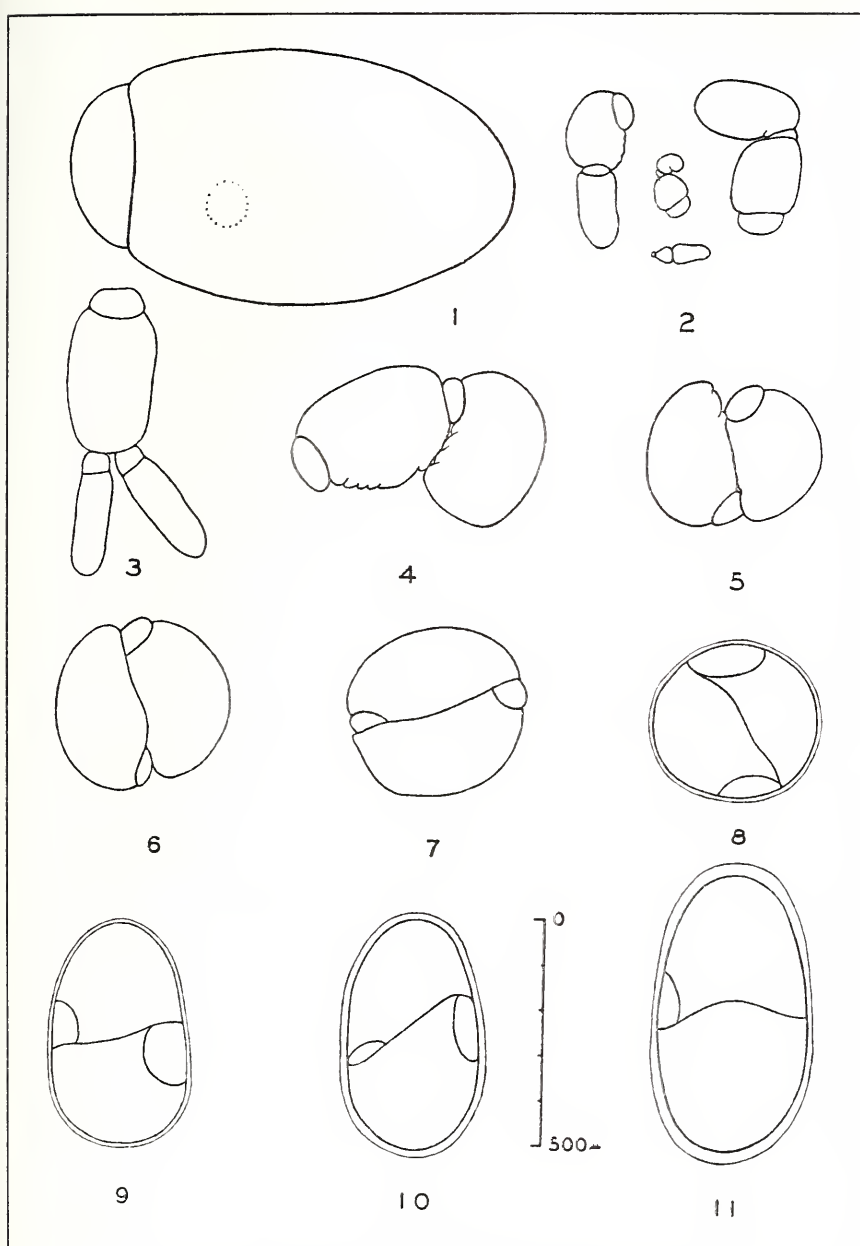


PLATE I

PLATE II

FIG. 12.—A cyst formed by three individuals of different size.

FIG. 13.—An unusually small cyst.

FIG. 14.—An unusually large cyst.

FIG. 15.—A cyst formed by two individuals of very unequal size.

FIGS. 16-20.—A number of successive developmental stages, as observed in a single cyst.

FIG. 16.—The cyst as it appeared after the gametes formed on the periphery.

FIG. 17.—About 30 minutes later. The gametes are seen to be separating from the residual mass.

FIG. 18.—About 20 minutes later. All the gametes have broken off and are floating freely in a liquid zone around the periphery. The two residual masses flow together.

FIG. 19.—About 10 minutes later. The residual masses are completely fused. The clear peripheral area containing gametes is still present.

FIG. 20.—About an hour later. The clear area disappears, and the contents appear uniformly granular.

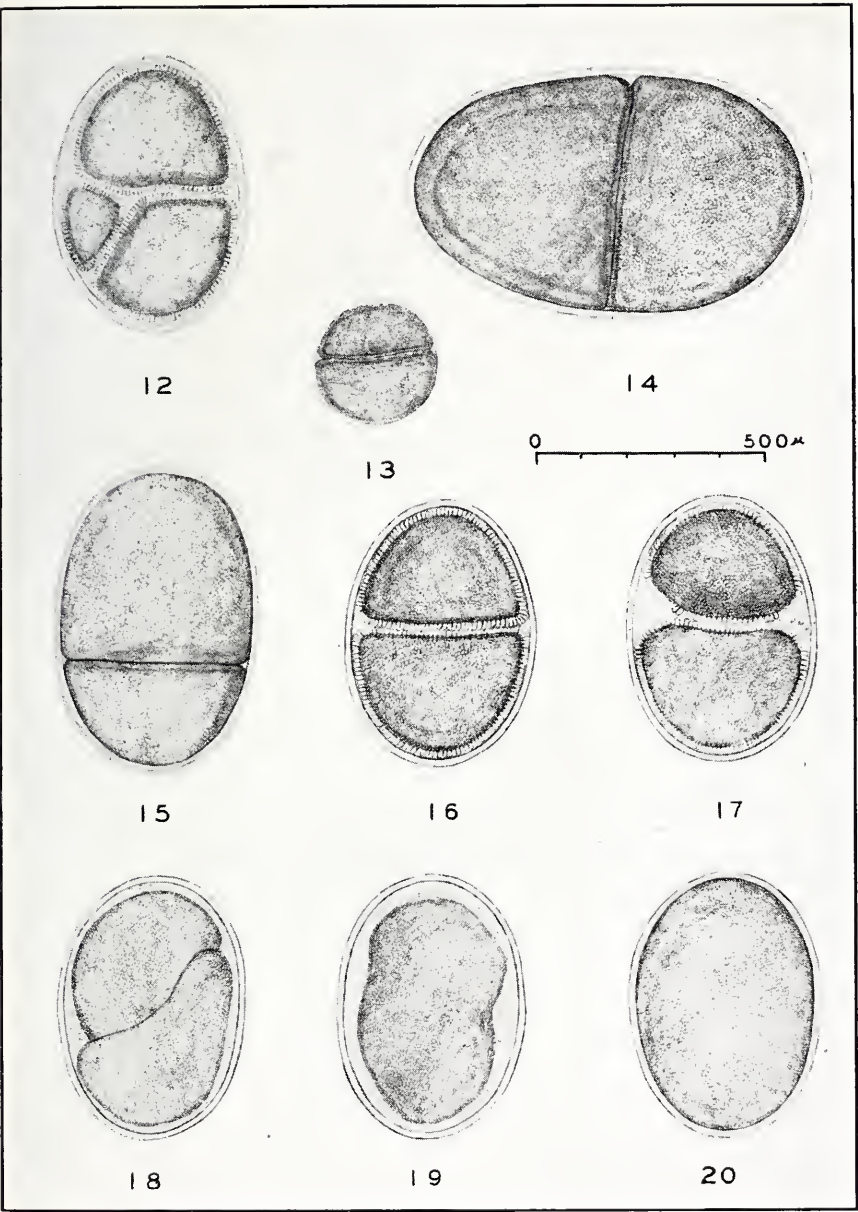


PLATE II

PLATE III

- FIG. 21.—Surface view of the basal disc of a sporoduct before the eversion of the latter. In life. $\times 413$.
- FIG. 22.—Optical section of a sporoduct showing expulsion of spores. In life. $\times 413$.
- FIG. 23.—Side view of basal disc just before eversion of the sporoduct. The basal disc is making its way through the cyst membrane and is elevated above the surrounding area. In life. $\times 94$.
- FIG. 24.—An incompletely everted sporoduct after the spores have been expelled through other sporoducts. A cavity in the cyst membrane due to lytic action in the region of the basal disc is shown. Neutral red. $\times 413$.
- FIG. 25.—An everted sporoduct. The basal region of the sporoduct and certain granules within the cyst are heavily stained with neutral red. $\times 413$.
- FIGS. 26-27.—Two optical sections of the same cyst membrane before and after the cyst was ruptured; showing great thickness and laminated appearance of the membrane after the cyst contents have escaped. In life. $\times 413$.
- FIG. 28.—A short chain of spores, showing how they are cemented together. In life. $\times 1730$.
- FIG. 29.—Optical section of a spore showing a mucoid sheath covering the true spore membrane. In life. $\times 1730$.
- FIG. 30.—A fresh spore with the mucoid sheath removed by acetic acid. $\times 1730$.
- Erratum:* The scale above Figs. 29 and 30 represents $10\ \mu$ instead of $20\ \mu$.

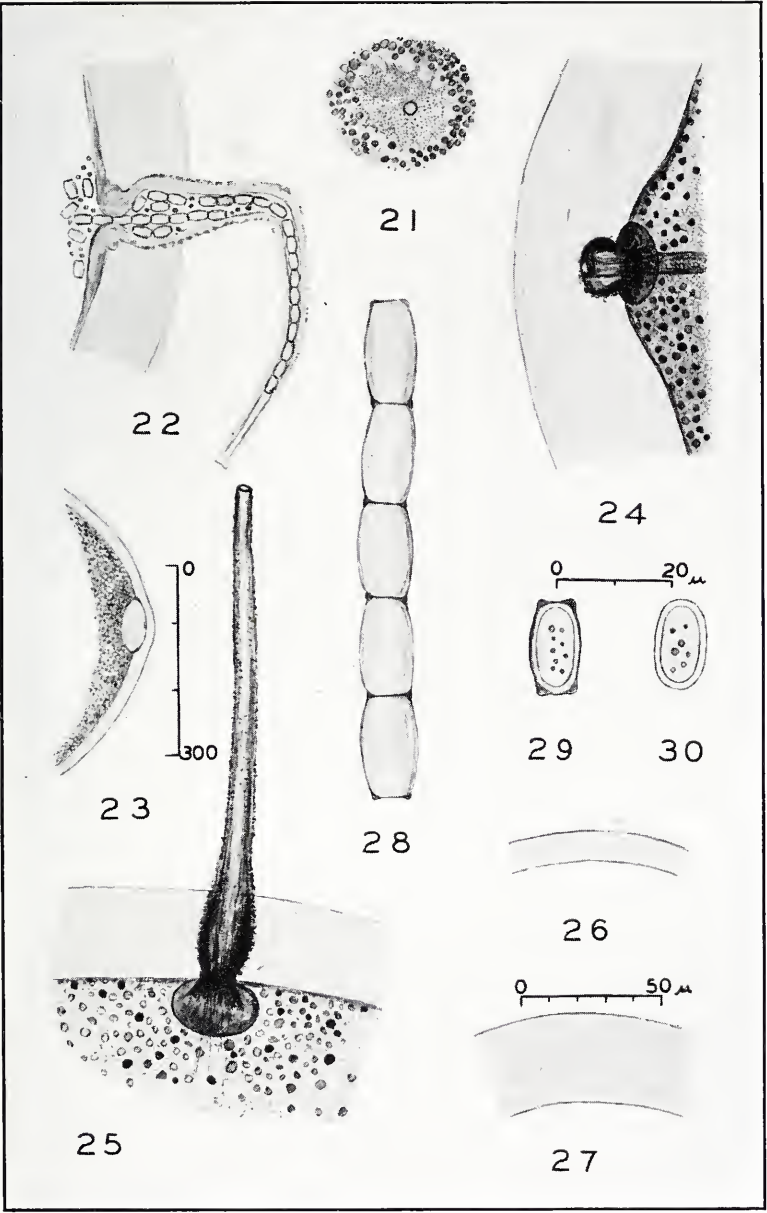


PLATE III

PLATE IV

- FIG. 31.—Photomicrograph of a cyst and discharged spores; showing extreme length of a single spore chain. In life. $\times 48$.
- FIG. 32.—Photomicrograph of a normal cyst a few hours before discharge of the spores. In life. $\times 48$.
- FIG. 33.—Photomicrograph of the same cyst the next day when the spores were discharged; showing great decrease in size due to contraction of the elastic cyst membrane. In life. $\times 48$.
- FIG. 34.—Photomicrograph of spore chain enlarged to show individual spores. In life. $\times 470$.
- FIG. 35.—Photomicrograph of mature cysts developed in a moist chamber. The chains of spores are arranged in large coils. In life. $\times 48$.
- FIG. 36.—Photomicrograph showing spores suspended on the surface film of an air bubble, indicating the presence of oily substance on the spores. In life. $\times 140$.

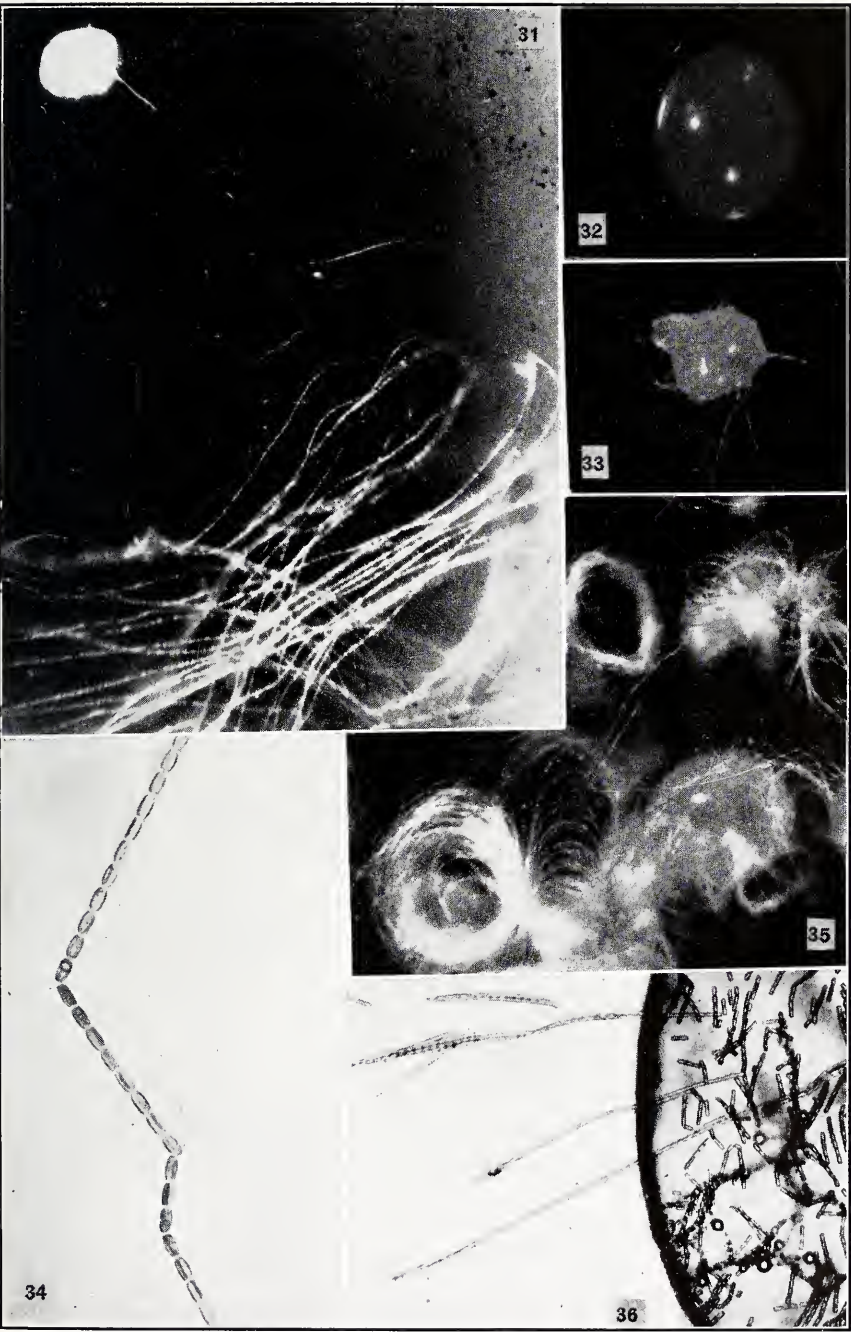


PLATE IV

PLATE V

- FIG. 37.—Anaphase in a pregametic division, showing three chromosomes which are characteristic in size and arrangement. The two short ones are 1 and 2, the long one is 3. S.-F.
- FIG. 38.—Later anaphase. S.-F.
- FIG. 39.—A telophase. S.-F.
- FIG. 40.—Interphase or early prophase. S.-F.
- FIG. 41.—Interphase and prophase nuclei. S.-F.
- FIG. 42.—A number of gametes just before they separate from the parent mass. S.-G.
- FIG. 43.—Gametes becoming rounded shortly after separating from the parent mass. S.-H.
- FIG. 44.—Two gametes after a period of growth; showing perinuclear vesicle. S.-H.
- FIG. 45.—Early stage in syngamy; showing characteristic manner in which the gametes come together. S.-H.
- FIG. 46.—Later stage in syngamy. The perinuclear vesicles have fused into one which contains the two pronuclei. S.-H.
- FIG. 47.—Later stage. The nuclear membrane of the posterior pronucleus has broken down and three chromosomes have emerged. Three prophase chromosomes are distinguishable in the other. Z.-H.
- FIGS. 48-49.—Peculiar appearance during syngamy in certain cysts. These are not in series with the other figures. S.-F.
- FIG. 50.—Later stage in syngamy; showing the chromosomes of the anterior pronucleus in the unravelling stage. With the breakdown of the nuclear membrane of the anterior pronucleus, the membrane of the common perinuclear vesicle becomes the membrane of the synkaryon. Z.-H.
- FIG. 51.—The two sets of 3 chromosomes from the two pronuclei lying side by side in the leptotene stage. Z.-H.
- FIG. 52.—Zygote showing 6 polarized leptotene threads in the synkaryon. Z.-H.
- FIG. 53.—Pairing of homologous chromosomes. The zygotene stage. Z.-H.
- FIG. 54.—The pachytene stage. The chromosomes are much shorter and thicker. Chromosome 3 (?) characteristically thicker than the others. S.-H.
- FIG. 55.—A stage in tetrad formation. The chromosomes are still shorter and thicker and are no longer polarized. S.-H.
- FIG. 56.—Three tetrads lying on the periphery of the nucleus. Z.-H.
- FIGS. 57-58.—Showing precocious disjunction in tetrads 1 and 2 while the nuclear membrane is still intact. Z.-H.
- FIG. 59.—Metaphase. The chromosomes are characteristically clumped together. Z.-H.
- FIG. 60.—A slightly later metaphase. Dyads 1 and 2 lie side by side while tetrad 3 is V-shaped. S.-H.
- FIG. 61.—Early anaphase. S.-H.
- FIG. 62.—Later anaphase showing disjunction in 3. S.-H.
- FIG. 63.—Anaphase. The tendency for the elements of the dyads to separate along the equational plane is seen during disjunction in 3. S.-H.
- FIGS. 64-65.—Later anaphase stages. A thread connects dyads 3. S.-H.

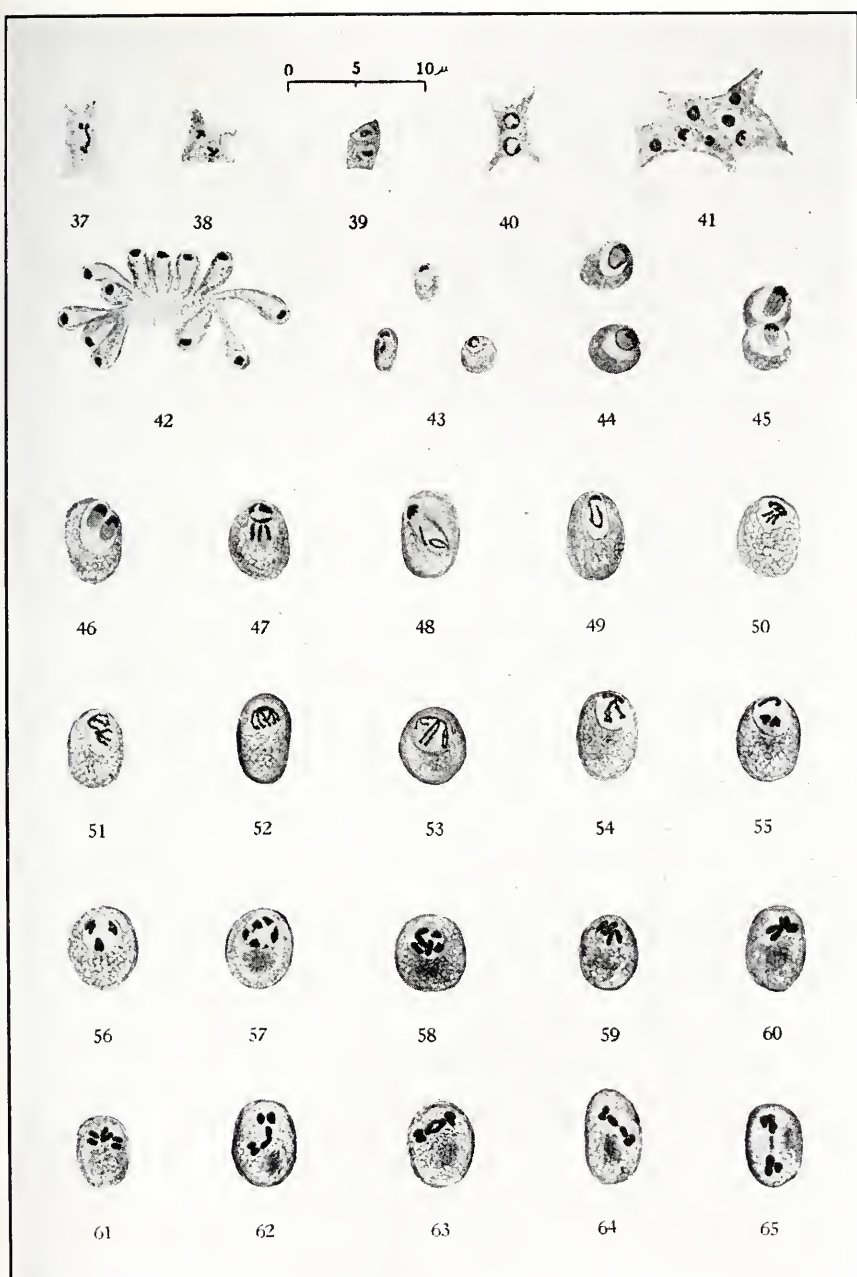


PLATE V

PLATE VI

FIG. 66.—Early telophase. S.-H.

FIG. 67.—Later telophase. S.-H.

FIG. 68.—Interphase. The nucleus is vesicular with chromatin granules scattered on a fine reticulum. This stage marks the end of the first metagamic (first meiotic) division. S.-H.

FIG. 69.—Prophase of second metagamic (second meiotic) division. Separation of the two elements in the dyads is seen to have occurred. S.-H.

FIGS. 70-71.—Metaphase (?). Long thin chromosomes lie in confused masses. S.-F. and Z.-H. respectively.

FIG. 72.—Metaphase in which the chromosomes are short and thick and lying in a clump. Z.-H.

FIGS. 73-74.—Anaphase stages. S.-F.

FIG. 75.—Early telophase. The chromosomes fuse to form an angle. S.-F.

FIG. 76.—Later telophase. The angles bend into circles which transform into vesicular nuclei. S.-F.

FIG. 77.—Interphase after second metagamic (second meiotic) division. The meiotic phenomena are now complete and each nucleus has a haploid set (three) of chromosomes. S.-F.

FIG. 78.—Prophase of the third metagamic division. Z.-H.

FIGS. 79-80.—Later prophases. The three chromosomes come to lie parallel with one another. S.-F.

FIG. 81.—Metaphase. The chromosomes lie in confused clumps. S.-F.

FIGS. 82-84.—Anaphase stages. S.-F.

FIG. 85.—Early telophase. The three chromosomes in each nucleus fuse into an angle. S.-F.

FIGS. 86-88.—Later telophase stages. The nuclei become circles. S.-F.

FIG. 89.—Each nuclear ring contains two granules, a large inner one and a small outer one. S.-F.

FIG. 90.—The nuclear rings elongate. S.-H.

FIGS. 91-92.—The final nuclear change is a transformation of the nuclear rings into long rods enlarged at the ends. S.-F.

FIGS. 93-95.—Sometimes the nuclei lie at or near the center of the spore, in which case the inner chromatin granule is small and the outer one is large.

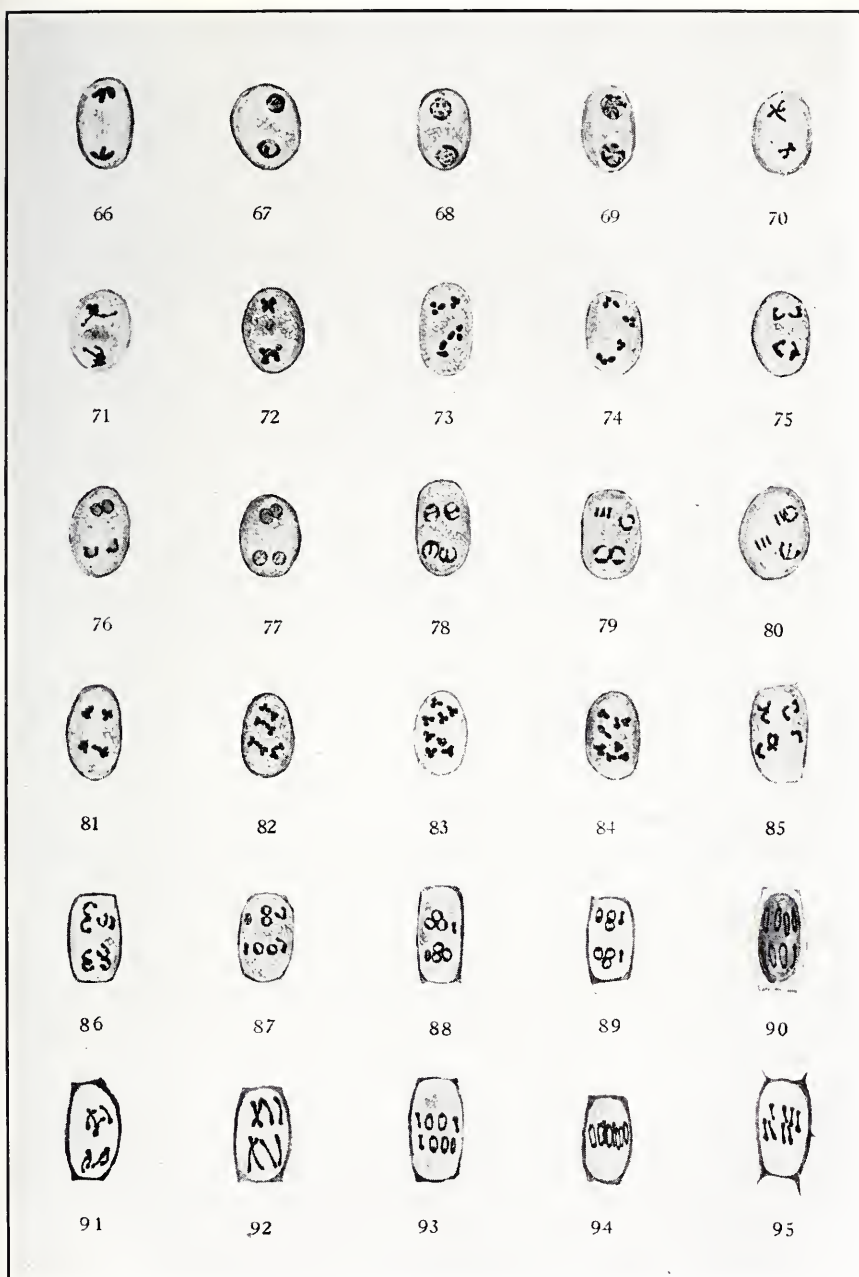


PLATE VI

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